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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EV 615196163 US

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Vladimir	SLEPUSHKIN	Damascus, Maryland
Laurent	HUMEAU	Germantown, Maryland

Additional inventors are being named on the _____ separately numbered sheets attached hereto

TITLE OF THE INVENTION (500 characters max)

AUTOLOGOUS T CELL MANUFACTURING PROCESSES

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

[X] The address corresponding to Customer Number: 25225

OR

[ ] Firm or Individual Name

Address

City	State	Zip
Country	Telephone	Fax

ENCLOSED APPLICATION PARTS (*check all that apply*)

[X] Specification *Number of Pages* 78
[ ] CD(s), Number of CDs _____
[ ] Drawing(s) *Number of Sheets* _____
[X] Other (specify) Return Receipt Postcard
[X] Application Data Sheet. See 37 CFR 1.76 (2 pages)

Application Size Fee: If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s)

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

[X] Applicant claims small entity status. See 37 CFR 1.27.

FILING FEE Amount ($): 100.00

[ ] A check or money order is enclosed to cover the filing fees.

[ ] Payment by credit card. Form PTO-2038 is attached.

[X] The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 03-1952
A duplicative copy of this form is enclosed for fee processing.

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[X] No. [ ] Yes, the name of the U.S. Government agency and the Government contract number are: _____

SIGNATURE _____ Date May 20, 2005

TYPED or PRINTED NAME Gregory P. Einhorn REGISTRATION NO. *(if appropriate)* 38,440

TELEPHONE (858) 720-5133 Docket Number: 397273001500

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

[Number 1 of 1]

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as Express Mail, Airbill No. EV 615196163 US, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Dated: May 20, 2005 Signature: _____ (Diane Blevins)

sd-260867

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AUTOLOGOUS T CELL MANUFACTURING PROCESSES

FIELD OF THE INVENTION

This invention relates generally to virology, cell biology and biotechnology. In particular, the invention provides novel processes for manufacturing autologous T cells, transducing T cells and expanding the transduced T cell population.

SUMMARY OF THE INVENTION

The invention provides processes for manufacturing autologous T cells and transducing T cells. Using the methods of the invention, high transduction levels are achieved when cells are cultured in solid plastic flasks versus plastic bags. In one aspect, for large scale transductions, 10-layer cell factories are used. In one aspect, 2-fold reduction of vector volume is necessary for efficient transduction of T cells on clinical scale. In one aspect, the vector (for the transduction of the T cell) is added twice with a 24 hour interval to further increase transduction.

In one aspect, in growing T cells lower concentrations of oxygen and slightly lower pH than in the regular media. The invention found that T cells expand better in the presence of lower concentrations of oxygen and slightly lower pH than in the regular media. In one aspect, N_2/O_2 90%/10% is used to culture T cells, in contrast to regular air (~20% of O_2). In one aspect, CO_2 concentration in air mixture is raised to 10%, up from usual 5%, to reduce pH. This changes in gas mixture allowed for higher expansion rates.

In one aspect, about 100 billion cells in the end of the expansion, perfusion of media is used. We found that in order to have about 100 billion cells in the end of the expansion, perfusion of media had to be used. In one aspect, a 50L perfusion bag is used, as previously used 20L bags were not able to support enough cells. In one aspect, perfusion is started as soon as cell concentration goes over $0.5x10^6$ cells/ml with the approximate speed of 3L/day. In one aspect, the speed is increased by approximately 2-fold next day with simultaneous increase of rocking speed and angle by 1 unit.

In one aspect, during the harvest T cells are cooled down to increase their viability after subsequent freezing and thawing. In some applications this needs to be done because the

harvest of large amount of cells takes long time and cells survive better at lower temperature. In one aspect, cells are transferred in smaller 10L bags and placed in the refrigerator for cooling. Refrigerated buffer should be used for washing cells.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

All publications, patents, patent applications, GenBank sequences and ATCC deposits, cited herein and the compact disc (submitted in duplicate) containing a sequence listing are hereby expressly incorporated by reference for all purposes.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings are illustrative of aspects of the invention and are not meant to limit the scope of the invention as encompassed by the claims.

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

Figure are enclosed. Like reference symbols in the various drawings indicate like elements.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides highly efficient methods and compositions related thereto for the stable transduction of cells with viral vectors and viral particles. The invention provides novel manufacturing facilities and manufacturing processes for cell processing of transduced cells, e.g., lentiviral vector-modified cells, such as autologous CD4+T cells, e.g., the exemplary VRX496-transduced CD4+T cells described herein.

In one aspect, the processes of the invention for manufacturing autologous T cells comprise isolation of lymphocytes, e.g., by frozen apheresis of blood, washing, e.g., in a CytoMate™ as described below, then CD4+ enrichment followed by CD8 depletion, followed by

transduction with virus, e.g., a lentivirus. Further processing is described below and illustrated in the figures herein.

In one aspect, the starting material for the production of Autologous VRX496-transduced CD4+ T Cells is peripheral blood mononuclear cells (PBMC). PBMC are obtained from an HIV-infected patient during leupkapheresis. The leukaphersis procedure can occur in a blood collection facility using an automated cell separator.

In one aspect, the cells are washed to remove plasma and magnetically labeled (incubated) with CD4 micobeads (Miltenyi Biotech, Germany), which have been developed for the separation of human cells based on the expression of the CD4 antigen. During Phase I clinical study, the starting material underwent ficoll density gradient separation by low speed centrifugation to remove plasma and then underwent COBE (Baxter) washing and resuspension in working buffer. The washed cell material was then incubated with CD8 high density microparticles (CD8-HDM nickel beads) (Biotransport) for subsequent magnetic separation using an Eligix Magnetic Cell Separation System.

For Phase II clinical study, cell washing to remove plasma can be performed using the CYTOMATE™ Cell Processing System (Miltinyi Biotech, Germany). The CYTOMATE™ Cell Processing System is a stand-alone, closed and automated device for washing and concentrating cellular products, and fluid transfer applications. It enables efficient cell washing with low cell loss and high viability. The system features a disposable tube set that creates a closed system fluid path for cell processing in a cGMP environment. It also makes fluid transfer flexible, fast and accurate. Solutions can be transferred to and from single or multiple containers, all within a closed system fluid path.

In one aspect, immune globulin solution (Immune Globubin Intravenous, USP, Grifols) is added to prevent non-specific cell binding during incubation of the added CD4+ microbeads (Miltinyi Biotech). In one aspect, the end product bag (CD4 microbead incubated cell suspension) is heat sealed and the bag is removed and placed under the biological safety hood.

In one aspect, an Eligix™ Cell Separation System is used for CD8 depletion. For Phase II clinical study, CD4+ positive selection can be performed via a CliniMACS™ magnetic cell separation system. This system uses a sterile CliniMACS™ disposable set consisting of (1) a

transfer pack container, (2) plasma transfer sets with female luer adapters for connection to a buffer bag and a cell suspension bag and (3) plasma transfer sets with female luer adapters for connection to a positive selection bag and a waste collection bag.

In one aspect, spiking of the sterile disposal sets' buffer and cell suspension lines to the respective bags can be done under a biological safety hood to maintain sterility. Once the phosphate buffered saline (PBS) buffer and cell suspension lines have been spiked, the disposable set can be attached to the CliniMACs™ and the CD4 magnetic labeled cell suspension can be run through the CliniMACS™. The collected positive fraction can be used to continue with the process.

In one aspect, PBS to X-VIVO-15 media (Cambrex; Walkersville, MD) exchange is accomplished via the CytoMate™. In one aspect, the end product bag is removed, heat sealed and placed under the biological safety hood. A transfer set with female luer adapter can be attached to the product bag and a 5 cc sample is obtained by syringe for QC testing for: the percentage of CD3+CD8+ cells and CD3+CD4+ cells, cell viability, cell number, and pre-expansion GIV Gag measurement. In one aspect, cell production stops until QC results are obtained. If the cells meet specification, production continues with cell transduction.

In one aspect, CD3/CD28 co-stimulation beads (Dynal beads, Oslo, Norway, coated with anti-CD3 (OKT3) and anti-CD28 (UPenn monoclonal antibody 9.3) are added to the CD4+ T Cell suspension followed by the addition of the VRX496 viral vector product. In one aspect, the whole mixture of CD4+ T cells, X-VIVO + 5% Human Serum Albumin, IL2, NAC, CD3/CD28 microbeads and VRX496 vector suspension (5% W/V) are added to a Nunc™ cell factory coated with RetroNectin (Takara Bio, Japan) and the cell factory put into a humidified, 37°C, 5% CO_2 incubator. VRX496 vector suspension (5% W/V) is once again added the next day. In one aspect, the cells are incubated with vector for 3 days, then transferred to WAVE™ cell bag and placed into a Wave™ Bioreactor (WAVE™ Biotech LLC, Bridgewater, New Jersey).

The WAVE bioreactor has a special rocking platform. The rocking motion of this platform induces waves in the culture fluid. These waves provide mixing and oxygen transfer, resulting in a perfect environment for cell growth that can easily support over 20 x 10^6 cells/ml. Tubing leads on the bags and a variety of connecting devices (connection will be via spike

connectors and welds produced via the Terumo Sterile Connecting Device) allow the cells to be grown in a closed system with minimal risk of contamination.

To remove vector, on the 4th day, the cells can be washed 2 times with X-VIVO 15 using the CytoMate™ cell washer.

Cell Expansion

The cultures are maintained for 7 to 12 days until it is time to harvest them. The cells are counted at least every other day and fresh medium is added to maintain the cells at an approximate density of 0.5 – 1.5 x 10^6 cells/ml. Antiretroviral drugs (Norvir, Abbot Laboratories, and Retrovir, GlaxoSmithKline) (1 μmol/L) are added to inhibit HIV replication while the cells are in culture. At about day 10, the cells are ready for harvesting. A post-expansion HIV Gag measurement is performed to insure that the post-expansion HIV copies are not greater than pre-expansion HIV copies. From the pre-harvest cells, a sample is taken to test for mycoplasma.

In one aspect, the CD3/CD28 microbeads are removed by passing the culture bag over a MaxSep™ magnet (Baxter). The beads are retained on the magnets and the cells are poured into another bag. The cells are assayed for residual beads.

TABLE OF CONTENTS

1.0 INTRODUCTION

This summary provides information describing a new manufacturing facility and manufacturing process for cell processing of lentiviral vector-modified cells, and specifically for Autologous VRX496-transduced CD4+T Cells.

During a Phase I clinical study, Autologous VRX496-transduced CD4+ T Cells were manufactured at the University of Pennsylvania (UPenn) Cell and Vaccine Production Facility (CVPF) by a proprietary process described under the University of Pennsylvania's BB-IND 6675, and made public in part through certain publications (Levine et al, 2002).

For a Phase II clinical study, the autologous VRX496-transduced CD4+ T cell product will be manufactured by VIRxSYS at its cell processing facility in Gaithersburg, Maryland. This proprietary process, although built upon the concepts known in the field at the time, has integrated a unique sequence of steps, materials, and equipment, that has allowed for the unprecedented expansion of over 100-fold of HIV-infected CD4 T cells modified by a lentiviral vector.

Submitted in this summary are:

- A description of the VIRxSYS facility, including the general facility layout,
- A description of the new manufacturing process, including the starting materials, in-process and release quality control testing, and stability, and
- Data demonstrating consistency in manufacture and comparability data to the UPenn CVPF process.

2.0 GENERAL FACILITY DESCRIPTION

The VIRxSYS establishment, consisting of approximately 25,000 square feet, is located at 200 Perry Parkway, Suite 1A, Gaithersburg, Maryland 20877.

The establishment contains the following functional areas:

- Female and Male Change Rooms (Purple): Rooms CR1 and CR2, respectively.
- Vector Production Suite (Dark Green): Clean Room, Gown Room, Materials Pass-Thru and De-Gown Room (Rooms P1, P2, P3 and P4, respectively).
- Cell Processing Suite (Dark Green): Clean Room, Gown Room, Materials Pass-Thru, De-Gown Room and Pass Box (Rooms P5, P6, P7, P8 and P9, respectively).
- Quality Control Testing Labs (Dark Red): Blood Processing, DNA Extraction, Replication Competent Lentivirus (RCL) Assays, QC Change Room, PCR A Lab, PCR B Lab, Raw Materials Lab and Micro Lab (Rooms Q1, Q2, Q3, Q4, Q5, Q6, Q7 and Q8, respectively).
- Warehouse Areas (Blue) Receiving/Storage/Shipping Area, Blood Processing, Cryo-Room/Packaging and Cold Room (Rooms, W1, W2, W3 and W4, respectively).
- Utility Areas (Pink): Mechanical Room, Autoclave Room, Telephone and Utility Room, and Janitorial (Rooms U1, U2, U3, U4 and U5, respectively).
- Quality Assurance and general office administrative areas (Light Green) and
- Research & Development Labs (Yellow).

The GMP manufacturing area and the quality control (QC) testing areas are contained and separated from the other establishment areas and from each other by physical barriers. The manufacturing area and QC testing areas are also restricted and controlled via card-key lock.

The intended uses of the two clean rooms are for clinical cGMP production of VRX496 lentiviral vector and the corresponding cGMP *ex vivo* transduction of patient's cells with this vector. The vector production clean room is a multiple product production facility and the cell processing clean room at the present time is intended only for the production of Autologous VRX496-transduced CD4+ T cells. Appropriate change-over procedures are in place between vector production and patient cell transductions. All patient cell products are tracked throughout the production process by a bar code system (see section 10.0).

To minimize any possibility of cross-contamination, vector production and cell processing operations do not share personnel and the areas are physically separated by location. Control is additionally maintained through written standard operating procedures (SOP) and personnel training on these procedures.

The Vector Production Clean Room Suite and the Cell Processing Clean Room Suite both have been designed for cGMP production and biosafety containment: Both Clean Rooms are designed as Class 10,000 (ISO Class 7) and Biosafety Level 2 (BSL-2) large scale.

The Clean Rooms have separate Air Handling Units (AHU). The Vector Clean Room AHU is a constant-volume, recirculating unit containing supply and return fans; pre 45% filter and 95% final filter; cooling coils, and a DX condensing unit. The AHU for the Cell Processing Clean Room supplies

100% outside air. The clean room finishes are constructed of smooth, hard, cleanable water- and chemical-resistant surfaces and the floors of seamless vinyl flooring with integral cove. Doors are constructed of galvanized steel with safety glass vision panels. Hardware features on the doors include kick plates, mop plates and door closures.

Similarly to the movement within the production areas, the movement between Quality Control (QC) Laboratories is controlled by SOP. The DNA Extraction Lab (Q2) is also separated physically from the PCR Labs (Q5 and Q6) to minimize any risk of contamination.

Construction of the VIRxSYS Cell Processing Clean Room Suite, Quality Control Testing Labs, Change Rooms and adjoining Warehouse was completed in August 2004. The Vector Production Clean Room Suite has existed since 2000, but was also modified in 2004 to separate out a materials pass-thru and gowning room into two separate rooms.

The proposed new cell processing facility design and vector clean room renovation was presented to representatives of the Center for Biologic Evaluation and Research, Office of Compliance and Biologic Quality and to representatives from the Office of Cellular, Tissue and Gene Therapy during a meeting (conference call) on November 19, 2003.

A U.S. Food and Drug Administartion (FDA) *ad hoc* inspection of the VIRxSYS Establishment was conducted on July 22, 2004 to determine compliance with the requirements of FDA Export Reform and Enhancement Act of 1996 (Export Reform Act). No deficiencies were noted and no FDA-483, Inspectional Observations, was issued. There have been no other FDA inspections conducted at this facility since construction has completed.

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The VIRxSYS Cell Processing Facility was initially registered with the FDA Baltimore District Office on August 24, 2004 for the production of Autologous VRX496-transduced CD4+ T Cells. The FDA assigned VIRxSYS Registration number is 3003569838.

3.0 NAME OF THE CELL PRODUCT MANUFACTURED

The name of the cell product manufactured and described under this amendment is Autologous VRX496-transduced CD4+ T Cells.

VRX496 is the laboratory code name for VIRxSYS' lentiviral vector, which contains a 937 nucleotide antisense sequence targeted to the Human Immunodeficiency Virus (HIV) envelope gene. The manufacture of VRX496 has been previously described under BB-IND 10606's initial IND submission, Serial No. 000, and in amendment, Serial No. 017 (VRX496 vector production scale-up).

Autologous VRX496-transduced cells are aliquoted into infusion bags (5 x 10^9 to 10^{10} transduced cells per 90 ml bag). The cells are suspended in infusible cryomedia consisting of:

- 31.25% Plasmalyte-A,
- 31.25% Dextrose (5%),
- 0.45% Sodium Chloride,
- 7.5% Dimethysulfoxide (DMSO),
- 1% Dextran 40, and
- 5% Human Serum Albumin.

4.0 STARTING MATERIALS

Process Step	Description	Test for	Test Article
2	Apheresed Product	Cell Concentration	PBMC
		% CD4	
		% CD8	
5	Positive Selected CD4+ T Cells	Cell Concentration	CD4+ T Cells
		% CD4	
		% CD8	
		Viability	
		Pre-HIV gag	
8	Cell Expansion	Cell Count	CD4+ T Cells

Attached is a list of starting materials used in the production of Autologous VRX496-transduced CD4+ T Cells.

All starting materials are received and inspected by VIRXSYS Materials Management personnel. The inspection includes completing the approved VIRxSYS "Raw Material Specification and Receiving Sheet." A VIRxSYS lot number is assigned, the package label examined and the Certificate of Analysis (C of A) reviewed for conformance to the approved VIRxSYS "Raw Material Specification and Receiving Sheet."

Quality Assurance (QA) reviews the "Raw Material Specification and Receiving Sheet" completed by Materials Management and either approves or rejects the material. If the material is "Approved" by QA it is labeled as "Approved" and moved by Materials Management personnel to an appropriate approved materials storage location within the Warehouse Area.

If a starting material is "Rejected" by QA, it is labeled as "Rejected" and segregated from the approved materials until a final disposition is determined, i.e., disposal, return to vendor, or transfer to R&D.

All starting materials are entered by QA into an Inventory Log. This log includes the VIRxSYS Lot Number assigned, quantity received, disposition, and expiration. Included in this inventory are in-house formulations such as buffers used in the manufacturing process or in QC test procedures. A monthly list of materials, which expire at the end of the month is generated by QA for Materials Management and/or Production to ensure their removal from the area and prevent inadvertent use.

Enclosed in Appendices A through S are the respective VIRxSYS Raw Material Specification and Receiving Sheet and representative C of A and/or U.S. Food and Drug Administration (FDA) approved labeling (i.e., immediate container label or package insert). Also included in the Appendices are Letters of Authorization authorizing cross-reference of relevant sections of the manufacturer's Investigational New Drug (IND) application or Master File (MF) for the monoclonal antibodies and the magnetic beads used in the process.

Reagent	Description of Use	Source of Materials (If of Human/Animal origin)	Reagent Quality	Vendor	Representative Certificate of Analysis or U.S. FDA Approved Labeling	Appendix
Albumin (Human), USP	Excipient of infused cell product	Human	U.S. Pharmacopeia/ U.S. FDA Licensed Biologic	Abbott	U.S. FDA Approved Product Label	A
CD4 Microbeads (Human)	Purification – Selection of autologous T cells	Humanized	Clinical Grade	Miltinyi Biotech	Vendor Certificate of Analysis; U.S. FDA Drug Master File	B
CD28 (9.3) Antibody	Cell Stimulation and Expansion	Murine/Human hybridoma	Clinical Grade	University of Pennsylvania	Certificate of Analysis; U.S. FDA Investigational New Drug Application	C
CD3/ CD28 Conjugated Magnetic Beads	Cell Stimulation and Expansion	Murine/Human hybridoma conjugated to Dynal Magnetic Microbeads	Clinical Grade	VIRxSYS	Certificate of Analysis	D

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Reagent	Description of Use	Source of Materials (If of Human/Animal origin)	Reagent Quality	Vendor	Representative Certificate of Analysis or U.S. FDA Approved Labeling	Appendix
Dimethysulfoxide (DMSO) (Cryoserv)	Cryomedia component – component of infusible cell media	Not applicable	Clinical Grade	Edwards Lifesciences	Certificate of Analysis	E
Human Sera, Type AB	Cell growth and expansion	Human	Clinical	Valley Biomedical	Certificate of Analysis	F
10% LMD in 5% Dextrose Injection (Low molecular weight Dextran for intravenous administration)	Cryomedia component – component of infusible cell media	Not applicable	U.S. FDA Approved Drug	Abbott	Certificate of Analysis	G
5% Dextrose and 0.45% Sodium Chloride Injection, USP	Cryomedia component – component of infusible cell media	Not applicable	U.S. FDA Approved Drug	Abbott	Certificate of Analysis	H
Immune Globulin Intravenous (Human) 5%	Purification – nonspecific blocker	Human	European Pharmacopeia; Clinical	Grifols	Certificate of Analysis	I
Interlukin-2 (Proleukin)	Cell growth and expansion	Human recombinant	U.S. FDA Licensed Biologic	Chiron	U.S. FDA Approved Package Insert	J
Magnetic Microbeads	Carrier for CD3 and CD8 purification antibodies	Not applicable	Clinical Grade	Dynal	Certificate of Analysis; U.S. FDA Drug Master File	K

Reagent	Description of Use	Source of Materials (If of Human/Animal origin)	Reagent Quality	Vendor	Representative Certificate of Analysis or U.S. FDA Approved Labeling	Appendix
(Multiple Electrolytes Injection, Type 1, USP) (Plasma-Lyte A Injection ph 7.4)	Included in Infusion to wash cells from bag. Included to be used in case of an emergency	Not applicable	U.S. Pharmacopeia/ U.S. FDA Licensed Biologic	Baxter	Certificate of Analysis	L
Norvlr (ritonavir oral solution)	Inhibitor of HIV protease in cell culture	Not Applicable	U.S. FDA Approved Drug	Abbott Laboratories	U.S. FDA Approved Product Label	M
Oclone OKT3 Sterile Solution (muromonrab-CD3)	Cell Stimulation and Expansion	Murine/Human hybridoma	U.S. FDA Licensed Biologic	Ortho	U.S. FDA Approved Product Label	N
Phosphate Buffered Saline Solution	Physiological agent for cells	Not Applicable	Clinical Grade	Baxter	Certificate of Analysis	O
Recombinant Human Fibronectin Fragment (RetroNectin)	Enhancement of transformation	Human	Clinical Grade	Takara Bio	Certificate of Analysis	P
Retrovir (zidovudine) IV Infusion	Pyrimidine mucleoside analogue against HIV in cell culture	Not Applicable	U.S. FDA Approved Drug	GlaxoSmithKline	U.S. FDA Approved Product Label	Q
Water for Injection, USP		Not Applicable	U.S. FDA Approved Drug	Baxter, Hospira Inc.	Certificate of Analysis	
X VIVO-15 W/O Gentamicin and phenol red w/5% Human Serum AB	Cell growth and expansion media	Human	Clinical Grade	Cambrex	Certificate of Analysis	R
VRX496 Lentiviral Vector	Gene-transfer agent	Not Applicable	Clinical Grade	VIRxSYS	Certificate of Analysis; U.S. FDA Investigational New Drug Application	S

5.0 PRODUCTION AND ROUTINE CONTROLS

5.1. Flow Diagram of the Process

Attached is a diagram of the cell processing purification (Figure 1) and manufacturing procedure (Figure 2).

Figure 1. Schematic representation of the purification process and associated equipment for isolation or enrichment of CD4 T lymphocytes from patient apheresis product, by using either CD8 depletion (CD4 enrichment) or CD4 positive selection (CD4 isolation)

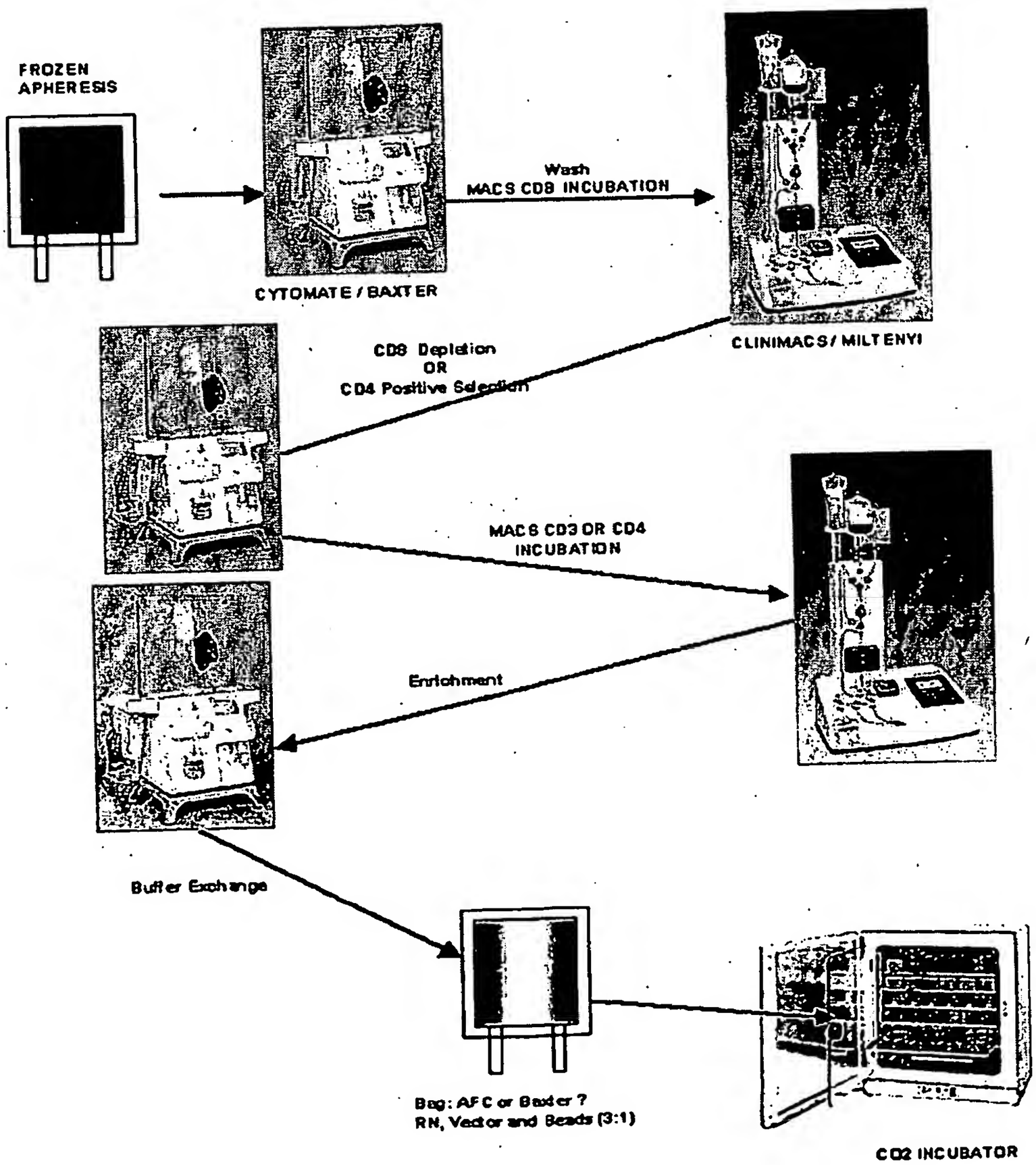
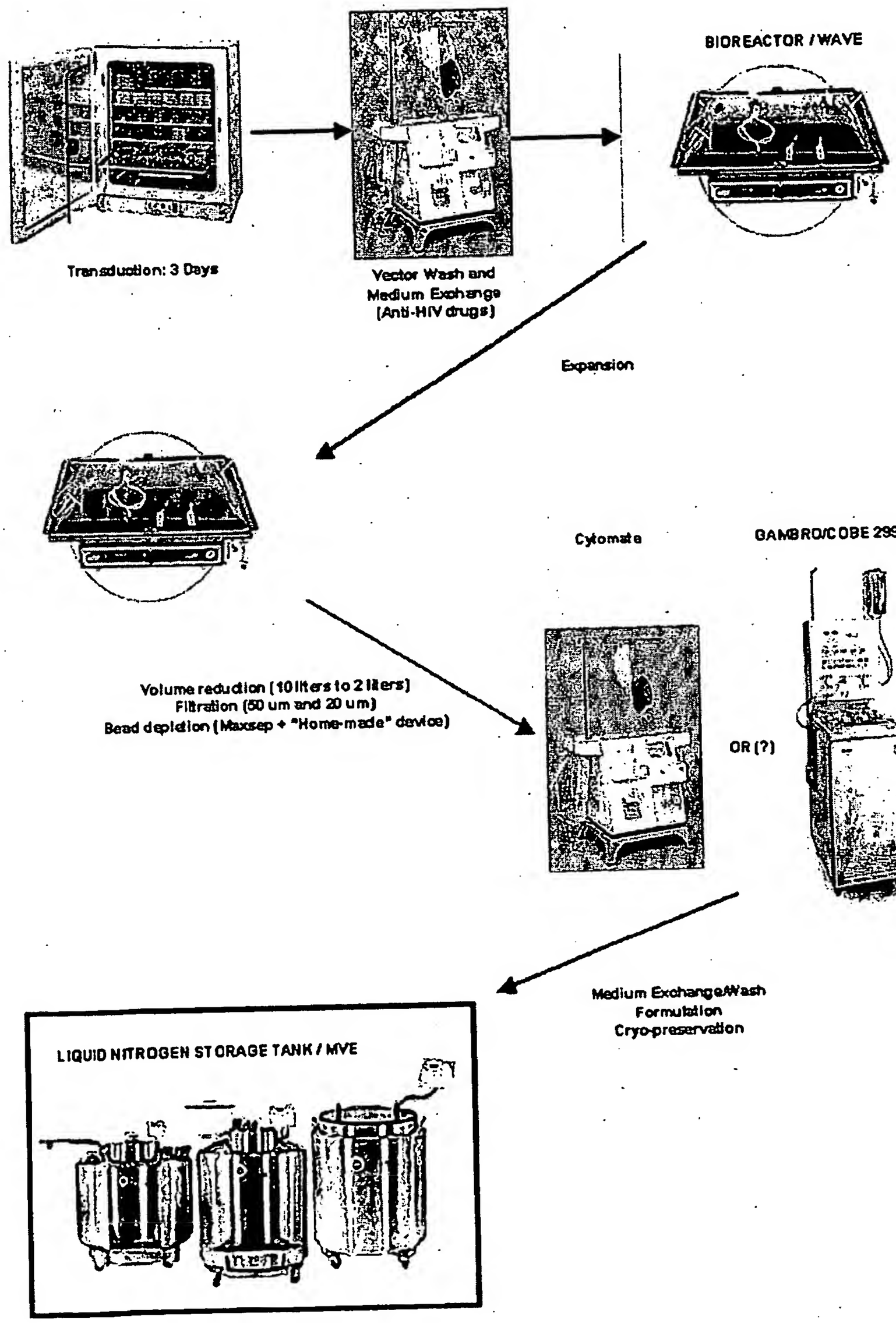


Figure 2. Schematic representation of the cell processing manufacturing including transduction, expansion and cryopreservation, with associated equipment. Product used in this figure comes from the initial isolation procedure described above in Figure 1.



5.2 Description of the Process

5.2.1 Method of Cell Collection

The starting material for the production of Autologous VRX496-transduced CD4+ T Cells is peripheral blood mononuclear cells (PBMC). PBMC are obtained from an HIV-infected patient during leupkapheresis. The leukaphersis procedure occurs in the blood collection facility using an automated cell separator (Cobe Spectra CS-3000, Baxter; Lakewood, CO).

Since up to eight cell infusions of approximately 5 x 10^9 to 10 to 10^{10} autologous VRX496-transduced CD4+ T cells each may be given to the subjects during the Phase II clinical study, approximately 3 to 4 blood volumes (15 L) of blood is required to be processed through the Cobe Spectra to collect sufficient PBMC (approximately 10 to 20 billion) to undergo the cell washing and selection procedures (i.e., purification) to result in the requisite number of CD4 T cells (i.e., approximately 1 to 2 billion) to begin the cell transduction and expansion processes. A single leupkapheresis procedure takes approximately 3 hours to complete.

In contrast, during Phase I clinical study, since each subject received only a single infusion of approximately 1 x 10^{10} autologous VRX496-transduced CD4+ T cells, the leukapheresed product collected was smaller, consisting of approximately 5 billion PBMC in approximately 70 mL.

The leukapheresed product will be shipped the day of its collection at ambient temperature from the respective blood center to VIRxSYS Corporation, Gaithersburg, MD by an air or land transport courier

(Calvalier Logistics Management, Dulles, VA) in accordance with IATA and DOD regulations. Transit time will be planned to insure that the leukapheresed product is received for processing by VIRxSYS production personnel no later than 24 hours after collection.

Since the product is both autologous and infectious, to insure product tracking control and to reduce the possibility of any product mix-up, each leukapheresed bag is labeled with:

- A unique lot number,
- The contained cell volume,
- A unique bar code label,
- The patient study ID (which includes identification of the study site),
- The patient initials, and
- The patient's birth date.

Additional precautions taken to reduce the possibility of mix-up are:

- A VIRxSYS written procedure allowing only 2 individual cell products to be manipulated at any given time within the cell processing clean room, and
- Product manipulations must involve different stages in the production process (e.g., CD4+ T Cell selection, vector removal or cell harvest).

  The exception to this policy is during the incubation and storage steps where as many as 80 separate products may be incubated in WAVE™ bioreactor and up to 30 stored in a freezer at any given time.

- Tracking of cell product throughout the manufacturing process with the aid of a bar code system.

5.2.2 VIRxSYS Receipt of Leukapheresed Product

Once the leukapheresed product is received at the VIRxSYS cell processing facility, Quality Assurance (QA) performs barcode scanning in the receiving room along with verification of the product bag label and records:

- Total volume received,
- Lot number on the bag,
- Time the bag was received, and
- Number of hours from the time of leukapheresis to the time of VIRxSYS receipt.

After QA releases the leukapheresed product, it is delivered by Materials Management personnel to the Cell Processing Clean Room (Class 10,000) (Biosafety Level 2 large scale). Production personnel take an approximate 5 cc sample of the cell product by syringe and add it to a vial for QC testing. QC tests for total CD4+ live cells, which should be $\geq 6 \times 10^8$ cells.

5.2.3 Plasma Washing and MACS CD4 Incubation

The cells are washed to remove plasma and magnetically labeled (incubated) with CD4 micobeads (Miltenyi Biotech, Germany), which have been developed for the separation of human cells based on the expression of the CD4 antigen.

During Phase I clinical study, the starting material underwent ficoll density gradient separation by low speed centrifugation to remove plasma and then underwent COBE (Baxter) washing and resuspension in working buffer. The washed cell material was then incubated with CD8 high density microparticles (CD8-HDM nickel beads) (Biotransport) for subsequent magnetic separation using an Eligix Magnetic Cell Separation System.

For Phase II clinical study, cell washing to remove plasma will be performed using the CYTOMATE Cell Processing System (Miltinyi Biotech, Germany). The CYTOMATE Cell Processing System is a stand-alone, closed and automated device for washing and concentrating cellular products, and fluid transfer applications. It enables efficient cell washing with low cell loss and high viability. The system features a disposable tube set that creates a closed system fluid path for cell processing in a cGMP environment. It also makes fluid transfer flexible, fast and accurate. Solutions can be transferred to and from single or multiple containers, all within a closed system fluid path

In addition, immune globulin solution (Immune Globubin Intravenous, USP, Grifols) will be added to prevent non-specific cell binding during incubation of the added CD4+ microbeads (Miltinyi Biotech).

The end product bag (CD4 microbead incubated cell suspension) is heat sealed and the bag is removed and placed under the biological safety hood. A QC sample of about 5 cc is taken for performing:

- Cell concentration and
- FACS analysis to determine the percentage of CD3+CD8+ and the percentage of CD3+CD4+.

Production stops until the QC results are received (approximately 30 minutes). The volume of the CytoMate end-product volume is calculated.

5.2.4 CD4+ Selection

As noted above, during Phase I clinical study, an Eligix™ Cell Separation System was used for CD8 depletion. For Phase II clinical study, CD4+ positive selection will be performed via a CliniMACS magnetic cell separation system. This system uses a sterile CliniMACS disposable set consisting of (1) a transfer pack container, (2) plasma transfer sets with female luer adapters for connection to a buffer bag and a cell suspension bag and (3) plasma transfer sets with female luer adapters for connection to a positive selection bag and a waste collection bag.

Spiking of the sterile disposal sets' buffer and cell suspension lines to the respective bags are done under a biological safety hood to maintain sterility.

Once the phosphate buffered saline (PBS) buffer and cell suspension lines have been spiked, the disposable set is attached to the CliniMACs and the CD4 magnetic labeled cell suspension is run through the CliniMACS . The collected positive fraction will be used to continue with the process.

The rationale for changing from a 2 step process during Phase I clinical study, i.e., CD8 depletion and CD3 selection, to a 1 step process during Phase II clinical study, i.e., CD4 selection, is for cell processing efficiency and to obtain a purer cell product.

5.2.5 Buffer to Media Exchange

PBS to X-VIVO-15 media (Cambrex; Walkersville, MD) exchange is accomplished via the CytoMate. The end product bag is removed, heat sealed and placed under the biological safety hood. A transfer set with female luer adapter is attached to the product bag and a 5 cc sample is obtained by syringe for QC testing for:

- The percentage of CD3+CD8+ cells and CD3+CD4+ cells,
- Cell Viability,
- Cell Number, and
- Pre-expansion GIV Gag measurement.

Cell production stops until QC results are obtained. If the cells meet specification, production continues with cell transduction.

5.2.6 CD4+T Cell Transduction

CD3/CD28 co-stimulation beads (Dynal beads, Oslo, Norway, coated with anti-CD3 (OKT3) and anti-CD28 (UPenn monoclonal antibody 9.3) are added to the CD4+ T Cell suspension followed by the addition of the VRX496 viral vector product. The whole mixture of CD4+ T cells, X-VIVO + 5% Human Serum Albumin, IL2, NAC, CD3/CD28 microbeads and VRX496 vector suspension (5% W/V) are added to a Nunc™ cell factory coated with RetroNectin (Takara Bio, Japan) and the cell factory put into a humidified, 37°C, 5% CO_2 incubator. VRX496 vector suspension (5% W/V) is once again added the next day. The cells are incubated with vector for 3 days, then transferred to WAVE™ cell bag

and placed into a Wave™ Bioreactor (WAVE™ Biotech LLC, Bridgewater, New Jersey).

The WAVE bioreactor has a special rocking platform. The rocking motion of this platform induces waves in the culture fluid. These waves provide mixing and oxygen transfer, resulting in a perfect environment for cell growth that can easily support over 20 x 10^6 cells/ml. Tubing leads on the bags and a variety of connecting devices (connection will be via spike connectors and welds produced via the Terumo Sterile Connecting Device) allow the cells to be grown in a closed system with minimal risk of contamination.

5.2.7 Washing to Remove Vector

On the 4th day, the cells are washed 2 times with X-VIVO 15 using the CytoMate cell washer.

5.2.8 Cell Expansion

The cultures are maintained for 7 to 12 days until it is time to harvest them. The cells are counted at least every other day and fresh medium is added to maintain the cells at an approximate density of 0.5 – 1.5 x 10^6 cells/ml. Antiretroviral drugs (Norvir, Abbot Laboratories, and Retrovir, GlaxoSmithKline) (1 µmol/L) are added to inhibit HIV replication while the cells are in culture. At about day 10, the cells are ready for harvesting. A post-expansion HIV Gag measurement is performed to insure that the post-expansion HIV copies are not greater than pre-expansion HIV copies. From the pre-harvest cells, a sample is taken to test for mycoplasma.

5.2.9 Washing, Volume Reduction and Formulation

The bag of cells are loaded on the CytoMate and the cells are washed out of the nutrient media and into an infusible cyromedia solution consisting of:

- 31.25% PlasmaLyte A,
- 31.25% Dextrose 5%,
- 0.45% NaCl,
- 5% Human Serum Albumin (HSA),
- 1% Dextran 40, and
- 7.5% DMSO.

5.2.10 CD3/CD28 Microbead Depletion

The CD3/CD28 microbeads are removed by passing the culture bag over a MaxSep™ magnet (Baxter). The beads are retained on the magnets and the cells are poured into another bag. The cells are assayed for residual beads.

5.2.11 Cryopreservation

The VRX496-transduced CD4+ T Cells are controlled-rate frozen. The cells cooling the product to cool at one degree (1°C) per minute until the product reaches the point of phase transition; then the freezing rate is increased until the temperature reaches -90°C.

5.2.12 Quality Control (QC) Release Testing

Samples are taken for QC Release Testing. The cell product is stored in the vapour phase of a liquid nitrogen freezer (set point of <-130° C) until completion of the QC testing.

5.2.13 Quality Assurance (QA) Release

After completion of QC testing, QA reviews all test results and if the release specifications have been met, authorizes the release of cell product for use in the clinical trial.

5.2.14 Storage

QA released cell product remains in liquid nitrogen storage until it is ready for shipping to the clinical site.

5.2.15 Shipping to Clinical Sites

Cell product is shipped to clinical sites at a temperature of ≤ 140° C in liquid nitrogen vapour shippers (Chart Inc., Marietta, Georgia, formerly MVE Cyrogenics) by contract transport courier (Cavalier Logistics Management, Inc., Dulles, Virginia) by their own freight truck or by commercial airline(s). These cryo-shippers have been validated to maintain their charge for 8-days.

6.0 QUALITY CONTROL (QC) TESTING

6.1 QC In-process Testing

In-process QC testing is performed as shown below. At this stage of development, these tests are done for information only.

6.2 QC Release Testing

The final cell product release tests and specifications are shown in the attached master Certificate of Analysis. The release tests are performed at the process steps and on the test articles presented below.

Process Step	Description	Test for	Test Article
4	Positive Selected Cell Product	Pre-expansion HIV gag to compare to post-expansion gag in step 10	Transduced Cells
10	Pre-harvested Cell Product	Mycoplasma	Culture Supernatant
		Bovine Serum Albumin (BSA)	Culture Supernatant
10	Harvested Cell Product	Microbead Removal	Transduced Cells
10	Post-Harvest Cell Product	Gtag	Transduced Cells
		E1A	
		Post-expansion HIV gag	
		VSVg RNA	Wash Supernatant
11	Cryopreserved Product	Sterility	Transduced Cells
		Endotoxin	Transduced Cells
1-Day Prior to Dosing	Infused Cell Product	Viablity	Transduced Cells

7.0 PACKAGING AND LABELING

Attached is a representative sample of the immediate container (infusion bag) and label for autologous VRX496-transduced CD4+ T Cells (*not included in this disclosure*)

8.0 QUALIFICATION OF THE PRODUCTION PROCESS

8.1 Summary of Major Manufacturing Changes Made Between Phase I and Phase II

Table 1 presents a summary of the major manufacturing changes made between Phase I and Phase II.

Table 1: Summary of Major Manufacturing Changes Made Between Phase I and Phase II

Manufacturing Change	Description
Change in Facility	Change from University of Pennsylvania Vaccine and Cell Production Facility to VIRxSYS Cell Processing Facility
Initial Wash of Apheresed Cell Product to Remove Plasma	From ficoll wash to CytoMate wash
CD4 Purification Process	Changed from CD8 depletion (Eligix) (CD8 antibody conjugated to nickel HDM) to CD4 selection (Miltinyi) (CD4 antibody conjugated to iron microbeads)
Cell Washing throughout Process	Changed from washes using Cobe (Baxter) to washes using Cytomate (Miltinyi)
CD3/CD28 co-stimulation micobeads	Presently produced by VIRxSYS using the same antibodies and beads used by the University of Pennsylvania Vaccine and Cell Production Facility for Phase I clinical study
Transduction	Performed in a cell factory rather than plastic bags on 2 days each with 5% suspension (W.V) of vector rather than 10% suspension (W/V) of the vector.
Cell Expansion	Presently incubating cells with WAVE incubator

8.2 Quality of the Apheresed Cell Product and Post-Positive Selected Cell Product: Phase I Versus Phase II

Table 2 provides a comparison of the CD4+ T Cell purity of the Phase I and Phase II starting material (i.e., post-washed apheresed product) and the post-positive selected cell product (i.e., cell product used to start the CD4+ T cell transduction and cell expansion).

As can be seen from these data, the Phase II cell production process results in a purer CD4+ starting material (average of 28.04% CD4 versus 14.58% CD4 for Phase I) and a purer CD4+ cell product to start the VRX496 transduction and cell expansion (average of 95.60% CD4 versus 36.82% CD4 for Phase I.

Additionally, the data show that the Phase 2 production process results in a post-positive selection cell product that is more consistent in purity than the product used in Phase 1 clinical study. This can be attributed to the single CD4+ positive selection step, whereas, the Phase I process used a 2 step process: CD8+ depletion and CD3+/CD4+ positive selection.

Table 2: Comparison of CD4+ T Cell Purity: Phase 1 Cell Product Versus Phase 2 Development Lots

Lot #	Apheresed Product		Post-Positive Selection	
	UPenn Phase 1 Cell Product	VIRxSYS Phase 2 Development Lots	UPenn Phase 1 Cell Product	VIRxSYS Phase 2 Development Lots
	Post-Wash	Post-CytoMate Wash and CD4+ Incubation	Post COBE Wash	Post-CytoMate Wash/ Media Exchange
1	11% Abs. 6.93×10^9	38.93% Abs. 6.124×10^9	56% Abs. 3.06×10^9 (52 % recovery)	97.62% CD4+ purity Abs. 3.419×10^9 (47.7% recovery)
2	15.9% Abs. 1.595×10^9	20.30% Abs. 3.00×10^9	52.2% Abs. 4.38×10^8 (48% recovery)	97.4% CD4+ purity Abs. 1.48×10^9 (40.5% recovery)
3	10.7% Abs. 1.015×10^9	24.9 % Abs. 3.71×10^9	23% Abs. 1.67×10^8 (26% recovery)	91.77% CD4+ purity Abs. 2.05×10^9 (48.8% recovery)
4	25.9% Abs. 2.533×10^9	None	33.4% Abs. 2.93×10^8 (30% recovery)	None
5	9.4% Abs. 7.86×10^8	None	19.5 % Abs. 1.77×10^8 (23% recovery)	None
Avg.% CD4	**14.58% (range 9.4 to 25.9%)**	**28.04% (range 20.3 to 38.93%)**	**36.82 (range 19.5 to 56%)**	**95.60% (range 91.77 to 97.62%)**

Table 3: Comparison of Phase I and Phase II Processes: CD4+ T Cell Expansion

Lot #	Phase I Cell Process (Clinical Trial Patient Lots)		Phase II Cell Process (Development Lots)	
	# of Cells at End-of-Culture ($x 10^6$ cells)	Fold Expansion	# of Cells at End-of-Culture ($x 10^6$ cells)	Fold Expansion
1	15811	65	52296	28.6
2	20638	40	104000	58.8
3	6785	25	96646	63.0
4	11454	32		
5	15212	66		
Avg	**13980**	**58.8**	**84314**	**50.1**

8.3 Comparison of VRX496 Transduction Efficiency: Phase I Versus Phase II

Table 3 presents a comparison of the VRX496 average vector copy number per cell between the Phase I cell products and the Phase II Development lots. As can be seen, average vector copy number remains essentially unchanged from Phase I, however, the average vector copy number among the Phase II Development lots are more consistent than those in Phase I.

Table 3: Comparison of Transduction Efficiency (Average VRX496 Vector Copy Number per Cell): Phase 1 Patient's Cells Versus Phase 2 Process Development Lots

Phase I Cell Product		Phase II Development Lots	
Subject Study ID	Results for Final Cell Product	Process Run #	Results for Final Cell Product
001-022 J-K	1.20	1	2.80
001-017 A-J	4.10	2	1.19

001-010 RAG	0.98	3	1.48
0001-001 JFJ	1.80		
001-002 R-B	2.3		1.82
Avg.	2.08		1.19 to 2.80
Range	0.98 to 4.10		

Specification = average of 0.5 to 5.0 VRX496 copies per cell.

8.4 Summary of Release Test Results for Phase II Development Lots

Table 4 presents a summary of the release test results for Phase II Development Lots 1, 2 and 3. All three development lots have met lot release specifications.

Table 4: Summary of Release Test Results for Phase II Development

Release Test	Specification	Development Lot #1	Development Lot #2	Development Lot #3
Vector Copy#	0.5 -5.0	2.8	1.19	1.48
Viability	≥ 70%	83.6	73.6	70.5
VSVg DNA	Mo copies	0	0	0
BSA				
E1A				
HIVgag				
Sterility				
Mycoplasma	Not Detectable	Pass	Pass	Pass
Endotoxin	< 3.5 EU/ml	0.06	0.06	0.06
Residual beads	< 100 per 3 x 10^6 cells	0	0	10
RCL				

9.0 STABILITY OF THE CELL PRODUCT

The VRX496-transduced CD4+ T cell products manufactured for the Phase 1 clinical trial were cryopreserved and stored at ≤ -80° C until scheduled for patient infusion. On the day prior to Infusion, a sentinel vial sample of cell product was thawed and measured for cell viability as part of the cell product release criteria. Each of the Phase 1 manufactured cell products had a cell viability of ≥ 70%. The longest time period of ≤ -80° C storage was 6-months. These data are supportive of the stability of the autologous VRX496-transduced cell product when stored at ≤ -80° C for up to 6-months.

To assess stability, a 24-months stability study of autologous VRX496-transduced CD4+ T cells will be performed on 6 autologous VRX496-transduced CD4+ T cell lots. These lots will be transduced with 2 different lots of VRX496 vector manufactured according to the existing manufacturing plan (i.e., 3 VRX496-transduced cell product lots per vector lot). The storage condition will be liquid nitrogen. Transduced cell product samples (15 ml) will be assayed at 3, 6, 12, 18 and 24 months. Time 0 data will be transduced cell product lot release data. Sufficient samples (20 bags per lot) will be collected at the end-of-cell processing to use for assaying at each time point. Assays will include: Appearance, Gtag copy number, cell viability, recovery, Intra-Cellular cytokine staining, sterility, and extra-cellular DNA concentrations. Interim reports will be written at the completion of testing for each time interval. A final report will be written at the end of the study. The QA department will be responsible for assuring the integrity of the data generated and for ensuring compliance with cGMP. All raw data, records and reports generated will be maintained at VIRxSYS corporation. Records to be

maintained will include storage conditions, storage unit validation and maintenance, sample preparation and raw assay data.

10.0 AUTOLOGOUS CELL PRODUCT TRACKING PROCEDURES

Autologous CD4+ T cells for 4 different patients may be processed concurrently. To protect these cell products from potential mix-up and contamination during this concurrrent manufacturing, these 4 cell products will be processed during different stages of production. Current good manufacturing practices will be followed. There are approved written cell processing procedures and all production personnel receive training on these procedures. Dedicated production equipment is used with procedures for production lot change-over. Critical equipment (incubator, freezers, HVAC) have been validated. Water for processing and all production materials used are obtained from approved vendors and according to established specifications. The following special controls to track patient cell products throughout the cell processing procedure have also been implemented:

Barcode System

- A custom designed barcode system tracks patient cells throughout the cell production and QC testing process, i.e., receipt, cell transduction, expansion, cryopresevation, storage, packaging and shipping.
- The barcode system provides an audit trail, user level access and full reporting capabilities.
- Prior to a patient cell product being processed or tested, production personnel scan both the material being processed or tested and the barcode affixed to to the batch production records or Quality Control (QC) test document for an identical match. If these do not

match, a warning is given on the computer screen. The individual scanning the material must then attest that a reconciliation was made and initials and dates the batchproduction records or QC test document.

Documentation

- Each patient's cell product lot is assigned a different color of documentation (i.e.; unique color for batch production records and QC test documents) to visually separate patient cell products during processing.

Segregation and Controls of Cell Product During Processing

- Only one production person is authorized to work with one patient cell product at any given time and all operations involving this cell product must be concluded before the next patient's cell product can be processed.
- All open air cell product manipulations are performed in a Class 100 Biological Safety Hood. Only cells from one patient are manipulated in the hood at any time.
- Patient cell product is incubated in WAVE™ bags and each patient's cell product lot has its own dedicated WAVE™ Incubator.
- Raw Materials such as buffers and reagents, which are placed into the hood are dedicated to one patient's cell product lot and discarded at the end of processing.

Segregation and Accountability of Cell Product During Storage

- Only one patient's cell product is cryopreserved at one time.
- During cryopreservation, each patient's cell product bags are protected within metal cassettes. After cryopreservation, these cassettes are connected by cable ties and stored segregated in freezer racks.
- Inventory of all stored cell product is maintained in the barcode system and by hardcopy documents.

May 20, 2005
Large Scale Processing for Cellular Therapies-Future Process

Overview of Current Process

The proposed cell processing procedures for the up-coming phase I/II clinical trials in VIRxSYS facility are summarized in detail in the attached document entitled, "**Large Scale Processing for Cellular Therapies-Current Process**". Briefly, the apheresis product will first go through a RBC depletion using the COBE 2991 cell processor (Gambro BCT). The resulting product will be then incubated with the Miltenyi anti-CD4 MACS and washed with the COBE 2991 cell processor. The anti-CD4 incubated product will be processed on the CliniMACS device, likely run twice for maximal yield.

The CD4 selected product will be immediately transduced with the vector in presence of stimulating beads in a RetroNectin coated bag. Transduction will be carried out for three days in a 37°C – 5% CO_2 incubator. Post-transduction cells will be washed using the Cytomate device (Baxter Oncology) before being expanded for a period of 8 to 10 days in the Wave Bioreactor. After expansion, stimulating beads will be removed using the Isolex 300i or Maxsep (Baxter Oncology, both), cell culture volume will be reduced, and cells washed using again the Cytomate, and prepared for cryo-preservation (formulation). Cryo-preservation will done with the Cryo-Med control rate freezer and cells will be stored in a vapor phase liquid nitrogen MVE tank. Overall, the process should take 11 to 13 days.

As proposed, the current cell processing procedures are time consuming, and expensive, but could be quickly implemented. The major cost identified in this process is the antibody selection step, and the major limitation for processing large number of patients is the 8 to 10 days expansion step.

Future Alternatives

Below are presented some technical alternatives aimed to simplify the current cell processing procedures, starting from the easiest to implement.

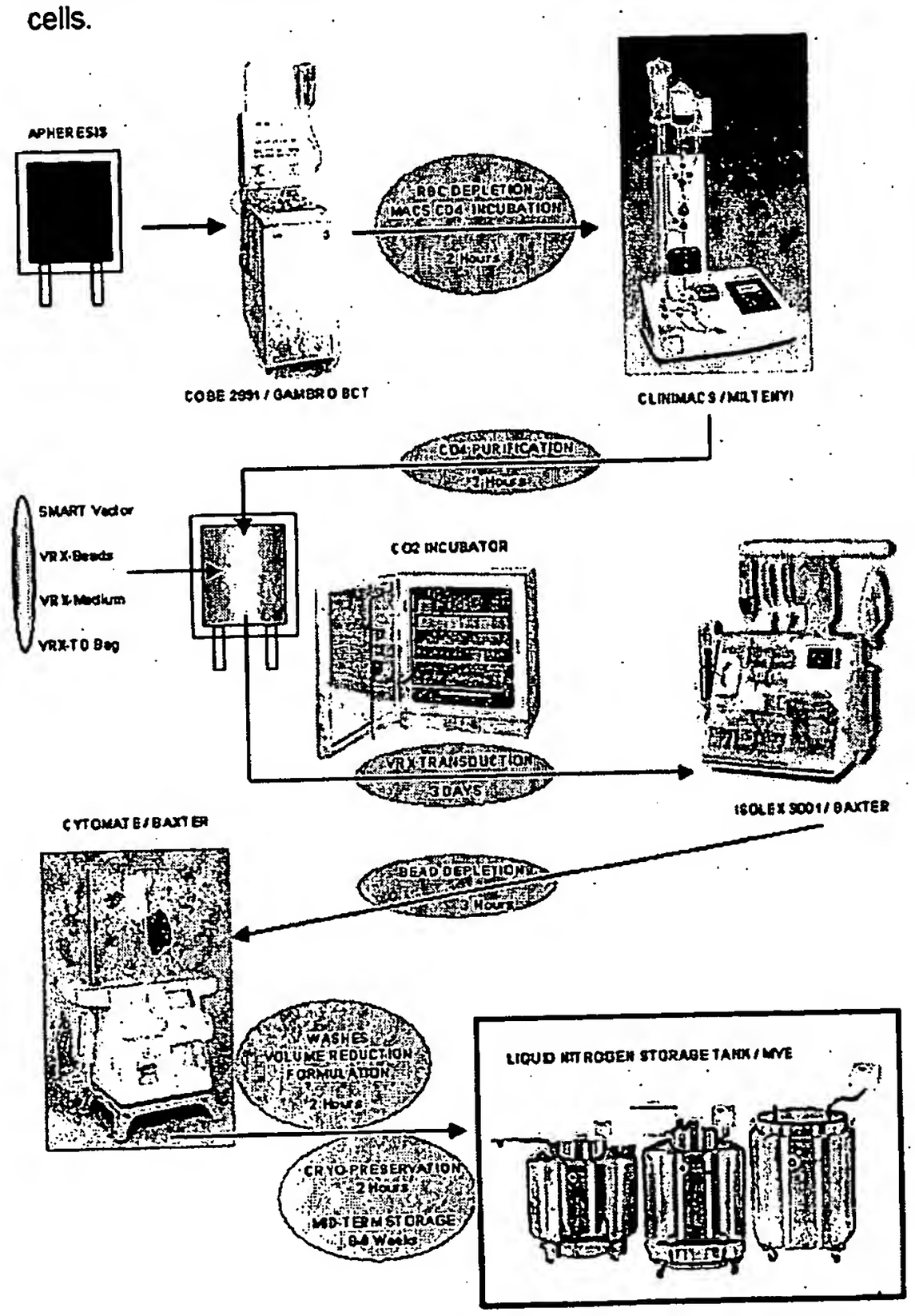
The first technical alternative concerns the length of expansion step, reducing it from 8 to 10 days to 0 as shown in **Figure 1**. Briefly, 3 day transduced cells will be directly processed for cryo-preservation (bead depletion, washes, and formulation). By reducing the time of product preparation from 11 to 13 days to 3 days, this will allow to process more product during the same period (4 vs. 1) and reduces as well associated expansion cost (Wave bioreactor and culture medium).

Associated with this first alternative, a limited or no purification step could be implemented, thus reducing the associated purification cost.

The second alternative would be the creation of a transduction kit, simple enough to be used at the clinical site without excessive and time-consuming manipulation procedures, as summarized in **Figure 2**. Briefly the fresh apheresis product will be directly incubated with a cocktail of antibodies that will link the RBC to unwanted cells, such as CD8+ and CD19+ lymphocyte (RosetteSep product, from Stemcell Technologies). Using the compact, automated and closed Sepax device (Biosafe), unwanted cells will sediment with the RBC during the centrifugation over a ficoll layer. Mononuclear cells will be collected, washed off ficoll with the same device, and transferred to a Teflon bag already containing the vector and the stimulating biodegradable nanobeads. Transduction will be

carried for 3 days in a 37°C – 5%CO_2 incubator, before before washed using again the Sepax device and being immediately re-injected to the patient.

Figure 1. Schematic overview of the "no expansion" cell processing procedure and associated equipment. This procedure is similar to that for the Current Process, but does not include the lengthy expansion step. CD4 positive selection is shown here, but either CD4 positive selection, or CD4 enrichment via depletion of non-CD4 cells can be used. "Smart vector" refers to a specially packaged lentiviral vector as described in US provisional number 60/585,464, that due to proteins incorporated in its envelope has an enhanced ability to bind, stimulate, and transduce cells.



The third alternative is summarized in **Figure 3**. This technical alternative is using only one processing step, the apheresis procedure, and is done at the clinical site in few hours. Briefly, the patient undergoes an apheresis procedure the same way he goes for the current and other proposed alternatives. The concentrated white blood cells are normally collected into a bag while RBC and plasma are continually re-infused to the patient. The collected white blood cells are then transduced in the collecting bag before being re-infused to the patient. No ex-vivo manipulations are required.

Limitations of Future Alternatives

ISOLATION

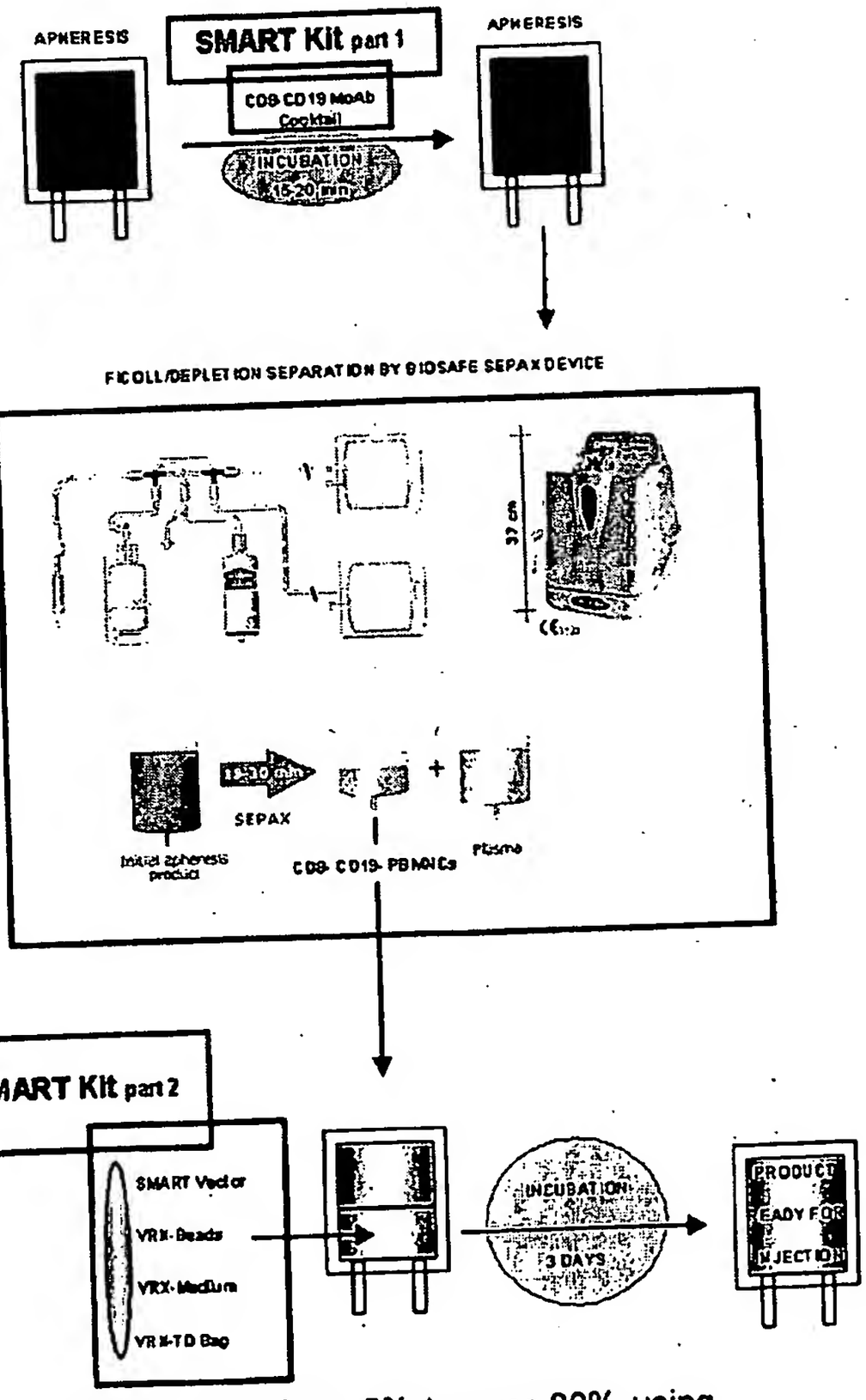
The 3-day purification/isolation alternatives have advantages and disadvantages. Doing just RBC depletion is the cheapest alternative; no clinical grade antibodies are required. The RBC depletion is not time consuming, and requires only one piece of equipment. However there is no control regarding the CD4 content.

The limited CD8 CD19 depletion alternative with the Sepax device needs only one piece of equipment, but might be more expansive due to the number of clinical antibodies

required for the depletion procedure (3) and the licensing of the Stemcell Technologies IP.

The current isolation procedure, CD4 positive selection, requires two pieces of equipment to be performed, and one clinical grade antibody (soon commercially available). The cost might be similar to the limited selection, but this procedure is more time consuming. However, the CD4 content is well controlled.

Figure 2. Schematic overview of the "no expansion" transduction kit for cell processing, and associated equipment. This procedure removes much of the equipment in Figure 1, and allows for a closed system of isolation and transduction of cells for decentralized distribution. Similar to Figure 1, this process does not contain an expansion step. The enrichment of CD4 cells is performed using the Rosette-Sep, which is a method for depletion of non-CD4 cells.



It was demonstrated one year ago that cells that went under only CD14 depletion, or CD14 and CD8 depletion, or CD14 depletion and CD4 purification had similar transduction levels, as assayed by flow cytometry. However the difference was in the level of expansion after a 7 days culture period.

EXPANSION

The current process has a culture period of 8 to 10 days. This process is based on clinical data from Carl June's group.

The proposed alternative is to reduce the time of expansion to the minimum necessary for the transduction. However few, if any, data are currently available to assess the in vivo expansion potential of 3 days manipulated T cells. Furthermore, the lack of an appropriate small animal model to assess the T cells reconstitution is a major limitation.

One proposed way to achieve in vivo expansion of transduced T cells would be to select them using the MGMT approach. Brian Davis demonstrated two years ago it was possible in vitro to select transduced primary CD4 T cells from 5% to over 80% using BG/BCNU drug treatment. Again, the lack of an appropriate animal model to assess the exact in vivo drug dosing is a major limitation.

Another option is the patient (*in vivo*) pre-conditioning with an anti-CD3 antibody before re-infusion of the manipulated cells. In vivo T cell depletion before transduced cell re-infusion could lead to a quick reconstitution of the T cell subset with the re-injected cells. Evaluation in large animal model or directly in phase I clinical trial seems to be the most appropriate way to go.

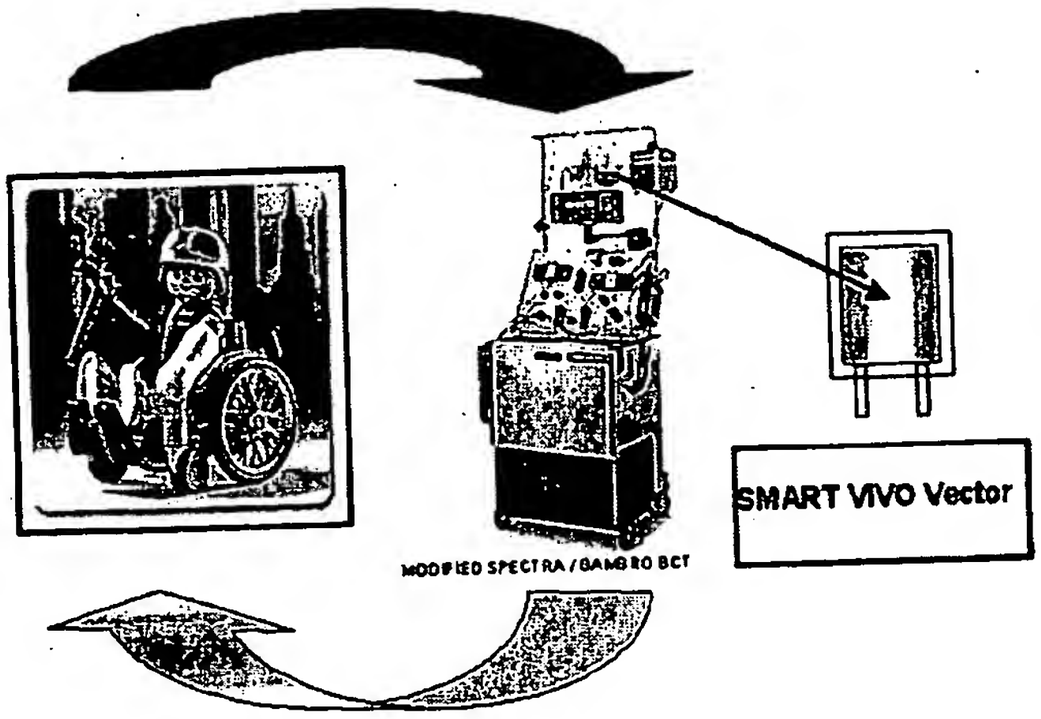
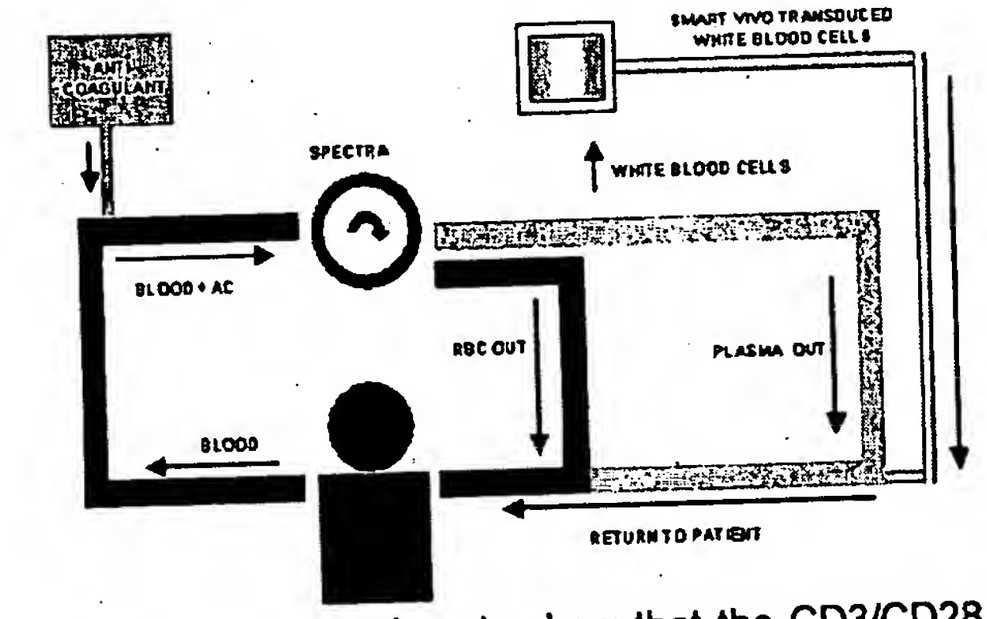


Figure 3. Schematic overview of the "in line" transduction process. This process uses the closed system of the apheresis machinery to perform any purification (this may or not be used), and transduction for direct reinfusion to the patient.



STIMULATION
The current stimulation procedure uses anti-human CD3 and anti-human CD28 murine antibodies coated to Dynal epoxy beads. Beside possible patent infringement for commercialization, one of the big hurdles of this stimulating system is the removal of the beads at the end of the culture. However, our experiments show that the CD3/CD28 stimulation is a key feature of our cell transduction protocol.

Four approaches can be developed, in the order of preference:
Using CD3 and CD28 antibodies linked on biodegradable nanobeads. This approach will not reduce the associated antibody stimulation cost but will reduce the product manipulation (no bead removal). Further development will need the participation of well established companies in the field of biodegradable nanoparticles (Miltenyi).
Using a superagonist anti-human CD28. This antibody has been shown to efficiently stimulate T cell expansion without the need of an anti-CD3 antibody. This superagonist antibody has been developed by a german company (licensing issue?). The limitations are similar to the current stimulation system.

Using the vector itself as T cell stimulatory proteins carrier. This approach is one of the most promising, but challenging. This approach does not require antibody production but will require extensive modification for the packaging cell line.

Using the Tetralink systen developed by Stemcell Technologies. This system bypass the need of bead support, thus avoiding the bead depletion step. This system however

requires the use of murine IgG1 monoclonal antibodies to be functional. Unfortunately, the selected CD28 clone from Diaclone is an IgG2a.

Specific Steps Towards Development of Future Process

SHORT TERM – 6 to 12 months -
Following the establishment of the various collaborations with the companies cited above, it is likely that the evaluation of the RosetteSep in the Sepax device can be performed with commercially available research grade antibodies. However, the source of clinical grade anti-human CD8 and CD19, IgG1 antibodies needs to be found. Diaclone is a likely source, if VIRxSYS is not implementing its own monoclonal antibody production in a near future.

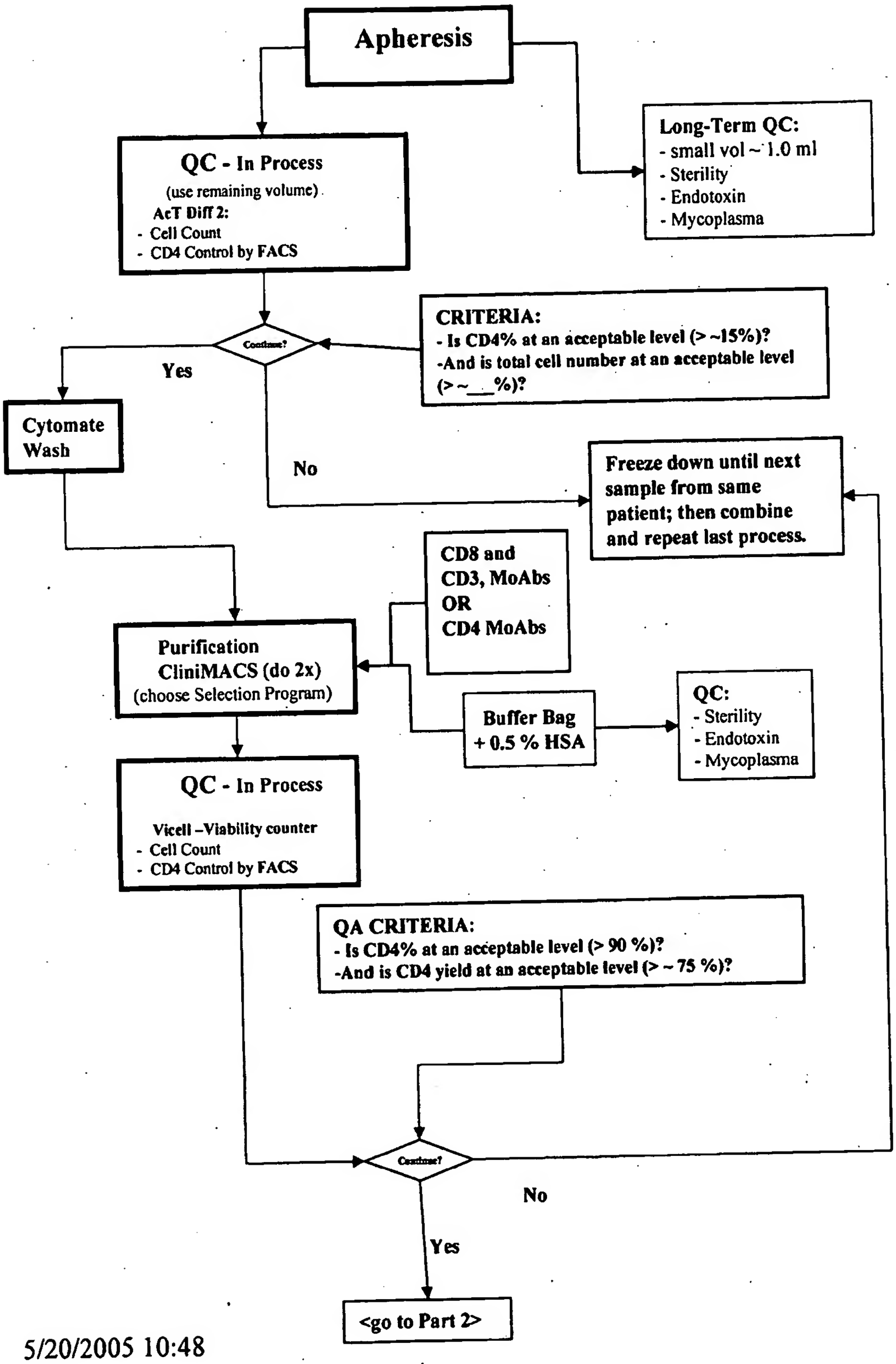
Development and evaluation of new T cell stimulatory system using Miltenyi biodegradable nanobeads.

Evaluation of the superagonist anti-human CD28.

MID TERM – 12 to 24 months –
Development and evaluation of new T cell stimulatory system using:
Stemcell Technologies tetralink system, with IgG1 CD3 and CD28 antibodies.

Evaluation of the vector envelop composition and packaging cell line modification, including the evaluation of the Maxygen's CD28BP.

LONG TERM – 24 and up –
Development of a kit as proposed in **Figure 3**, assuming clinical grade vector produced from modified packaging cell line using biodegradable nanobead free stimulating system, and safety/efficacy from large animal model for 3 days culture period and in vivo T cell reconstitution.



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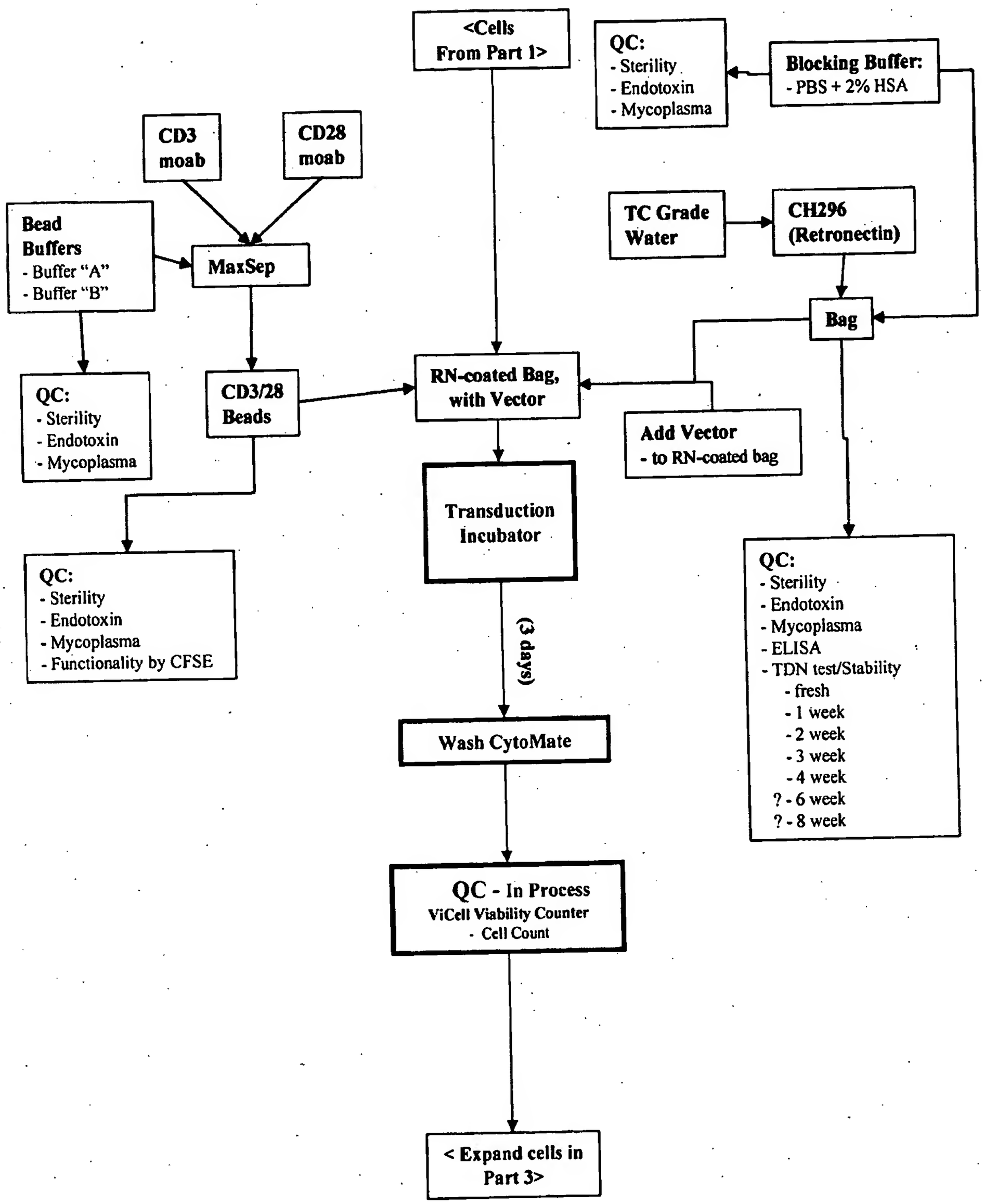

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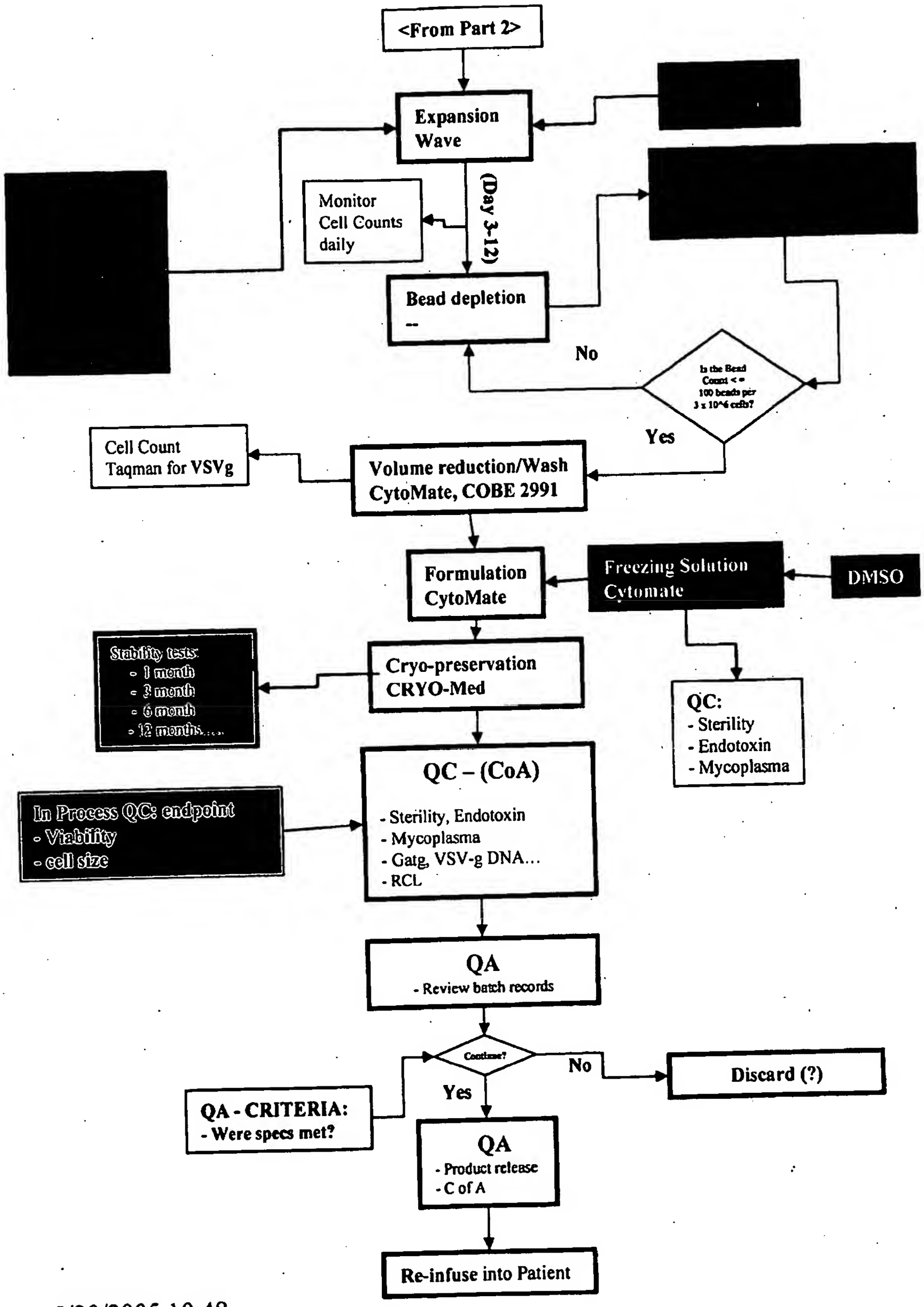

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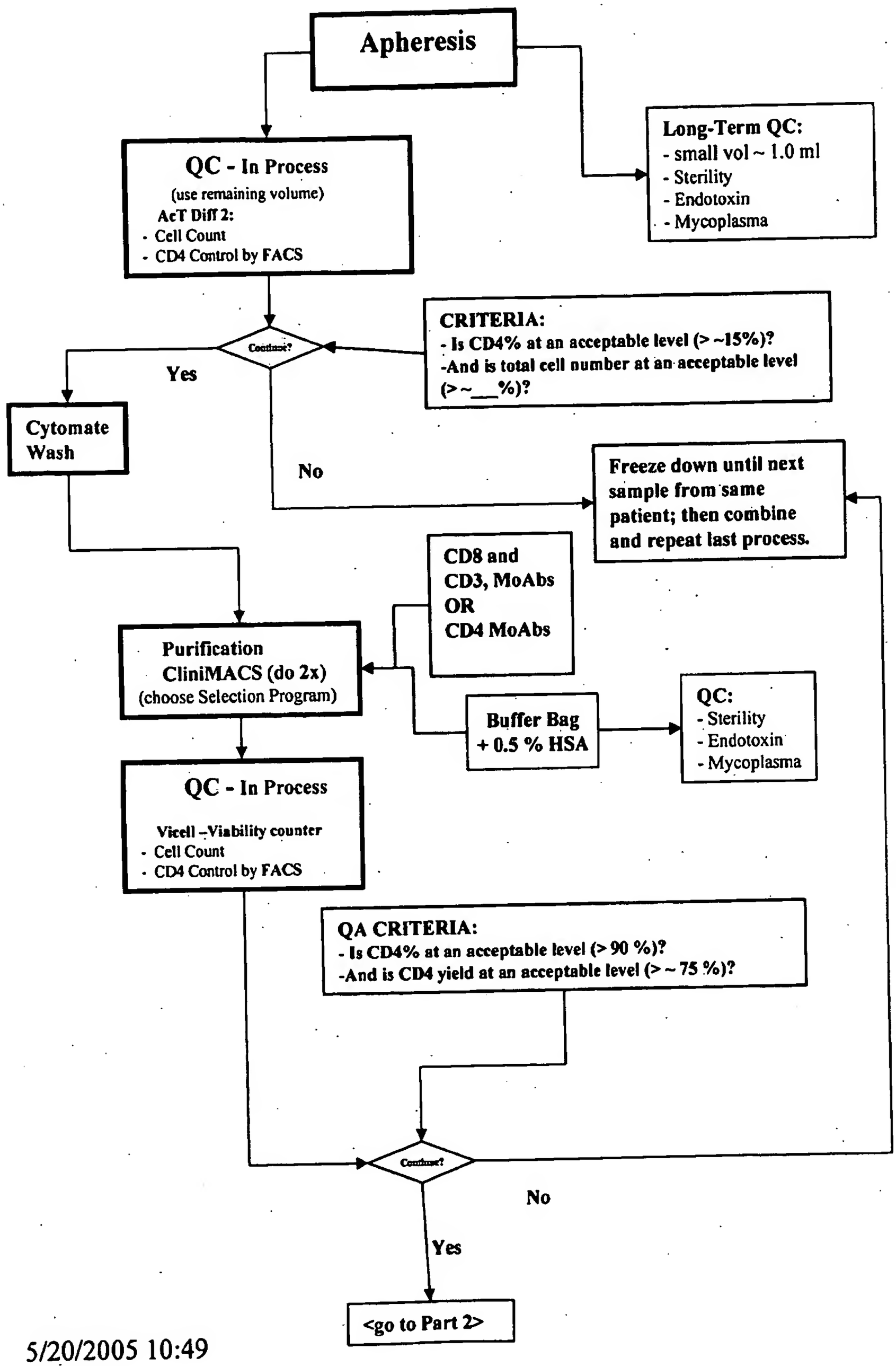
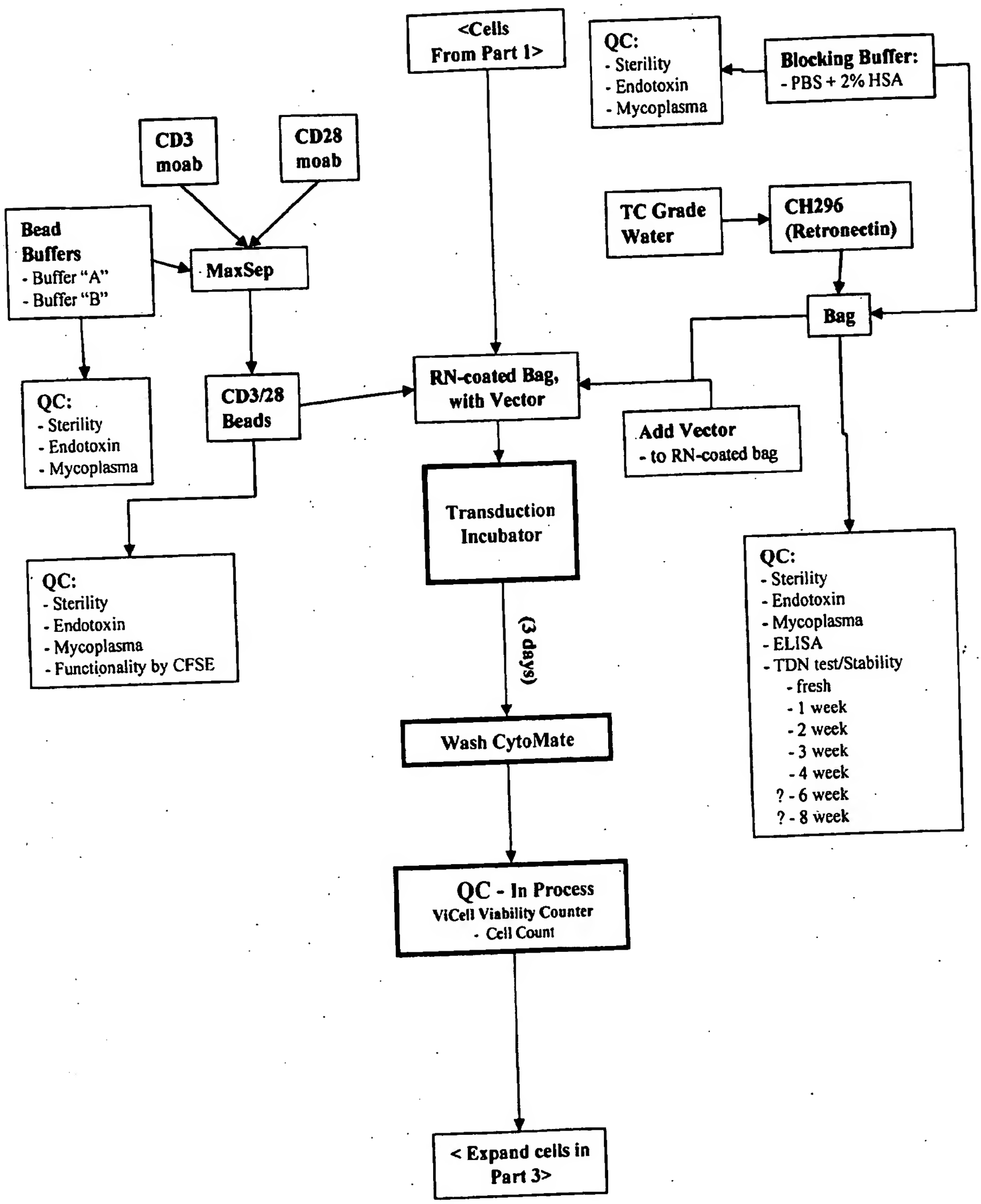
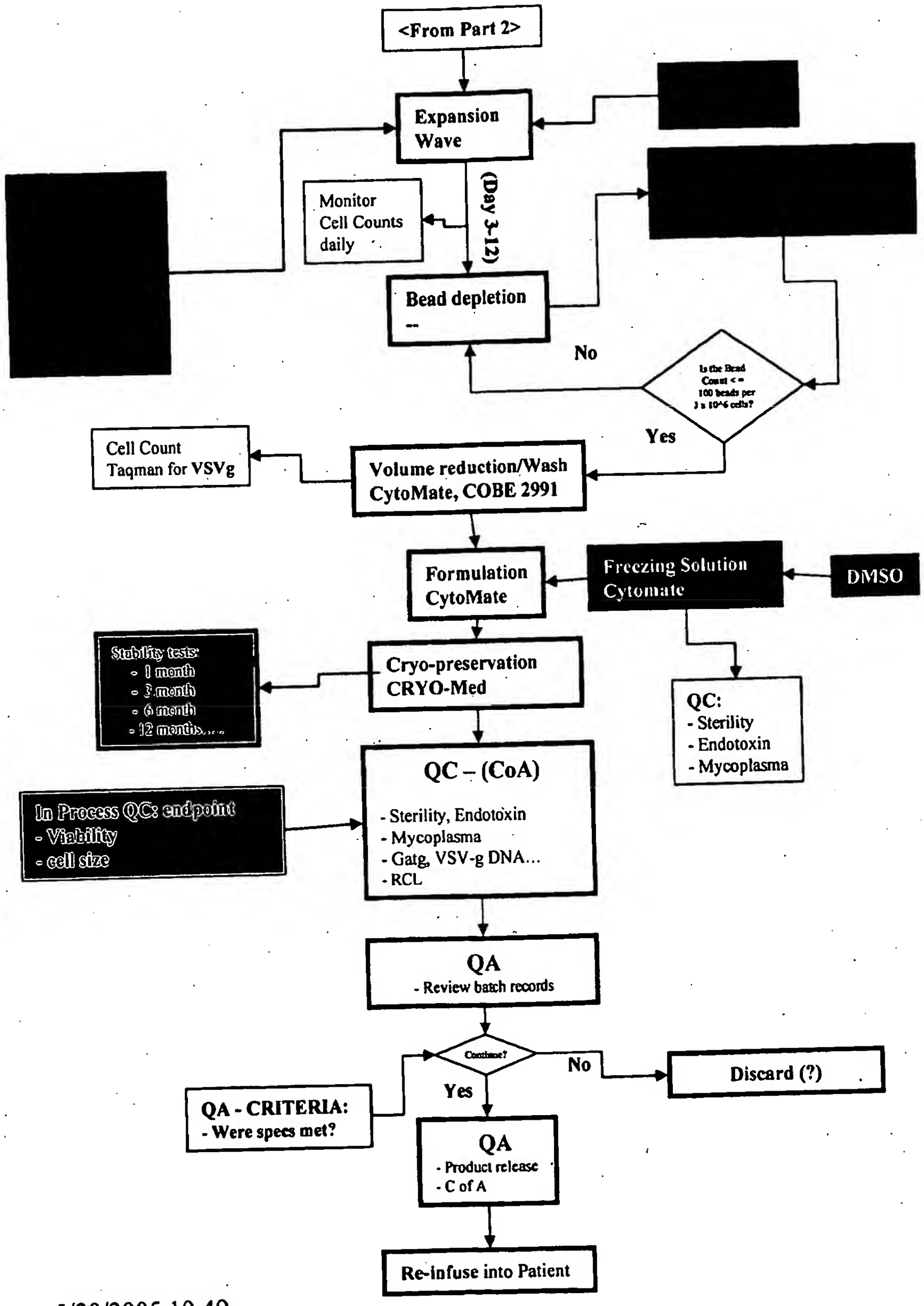

Apheresis
Long-Term QC:
- small vol ~ 1.0 ml
- Sterility
- Endotoxin
- Mycoplasma
QC - In Process
(use remaining volume)
AcT Diff 2:
- Cell Count
- CD4 Control by FACS
Continue?
CRITERIA:
- Is CD4% at an acceptable level (> ~15%)?
-And is total cell number at an acceptable level (>~___%)?
Yes
No
Cytomate Wash
Freeze down until next sample from same patient; then combine and repeat last process.
CD8 and CD3, MoAbs OR CD4 MoAbs
Purification CliniMACS (do 2x)
(choose Selection Program)
Buffer Bag + 0.5 % HSA
QC:
- Sterility
- Endotoxin
- Mycoplasma
QC - In Process
Vicell –Viability counter
- Cell Count
- CD4 Control by FACS
QA CRITERIA:
- Is CD4% at an acceptable level (> 90 %)?
-And is CD4 yield at an acceptable level (> ~ 75 %)?
Continue?
No
Yes
<go to Part 2>


<Cells From Part 1>
QC:
- Sterility
- Endotoxin
- Mycoplasma
Blocking Buffer:
- PBS + 2% HSA
CD3 moab
CD28 moab
Bead Buffers
- Buffer "A"
- Buffer "B"
MaxSep
TC Grade Water
CH296 (Retronectin)
Bag
QC:
- Sterility
- Endotoxin
- Mycoplasma
CD3/28 Beads
RN-coated Bag, with Vector
Add Vector
- to RN-coated bag
Transduction Incubator
QC:
- Sterility
- Endotoxin
- Mycoplasma
- Functionality by CFSE
(3 days)
QC:
- Sterility
- Endotoxin
- Mycoplasma
- ELISA
- TDN test/Stability
- fresh
- 1 week
- 2 week
- 3 week
- 4 week
? - 6 week
? - 8 week
Wash CytoMate
QC - In Process
ViCell Viability Counter
- Cell Count
< Expand cells in Part 3>



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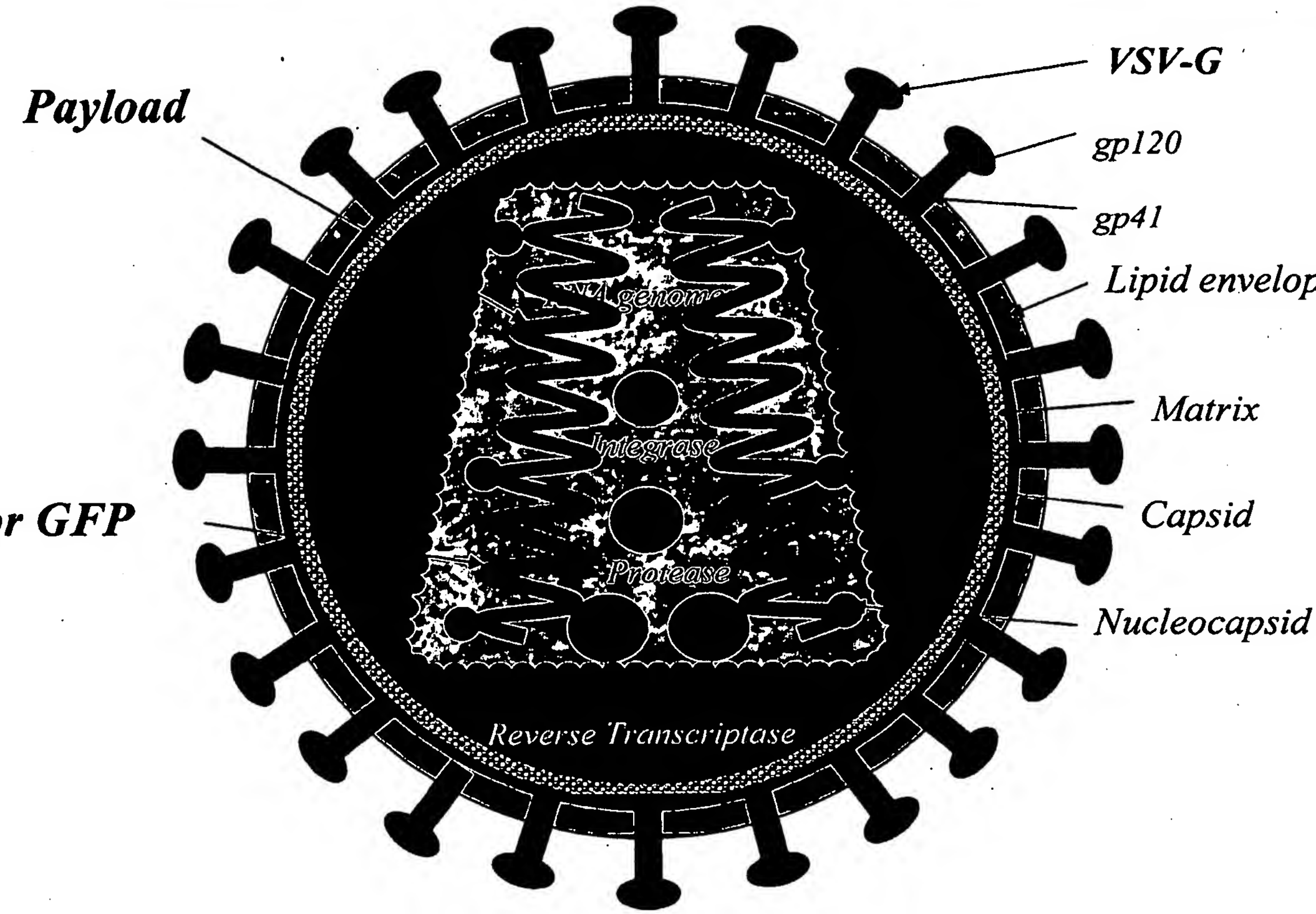
50

Lentiviral Vector Construction Process



Gtag or GFP

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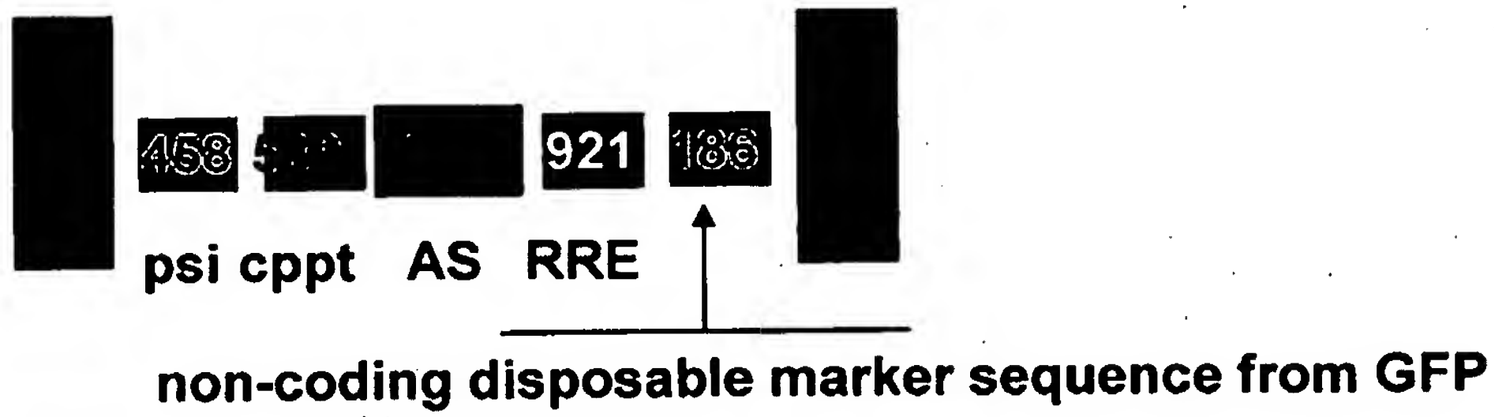
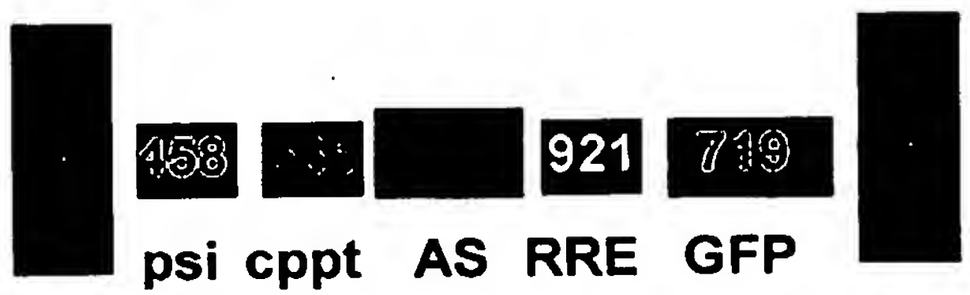
VIRxSYS

VIRxSYS Packaging System

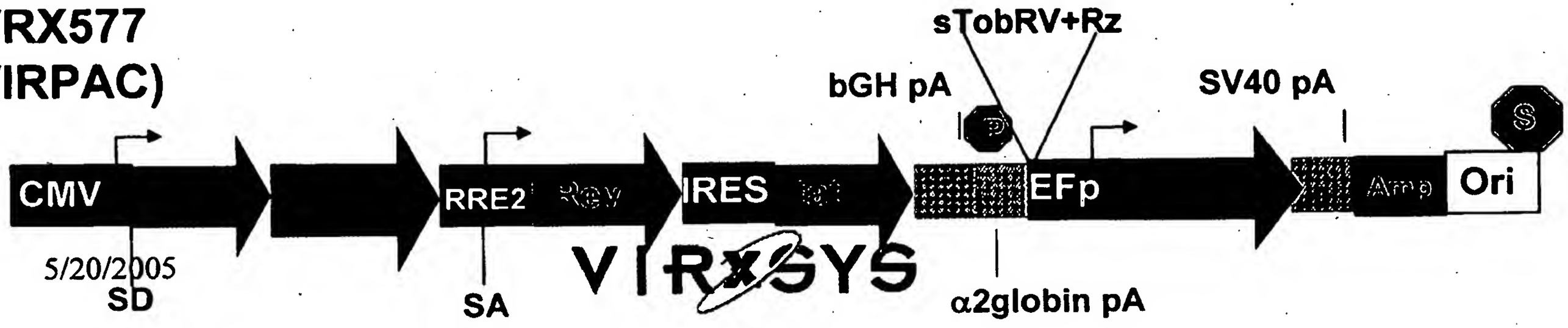
VRX496
(4344bp)



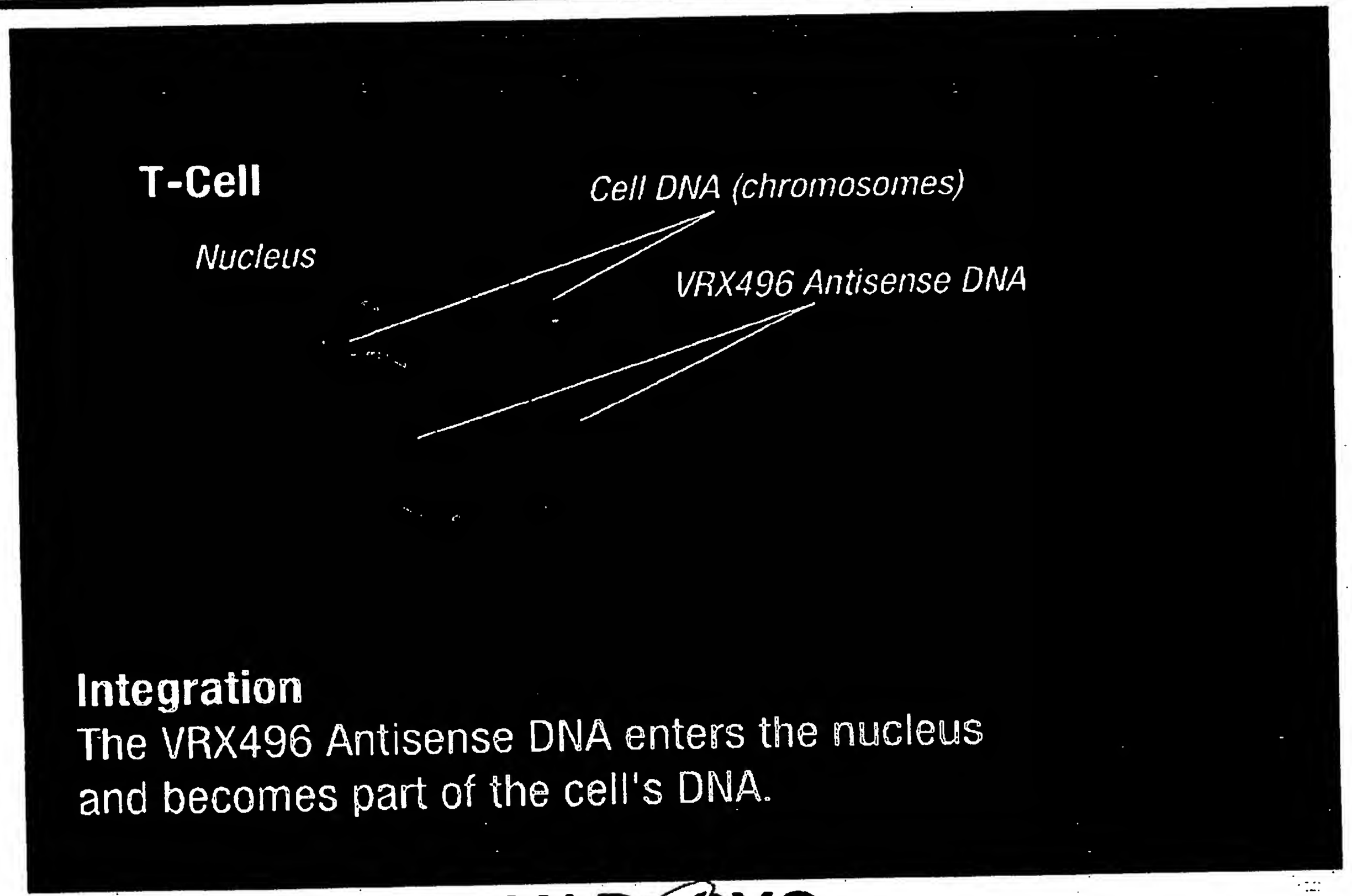
VRX494
(4877bp)



VRX577
(VIRPAC)



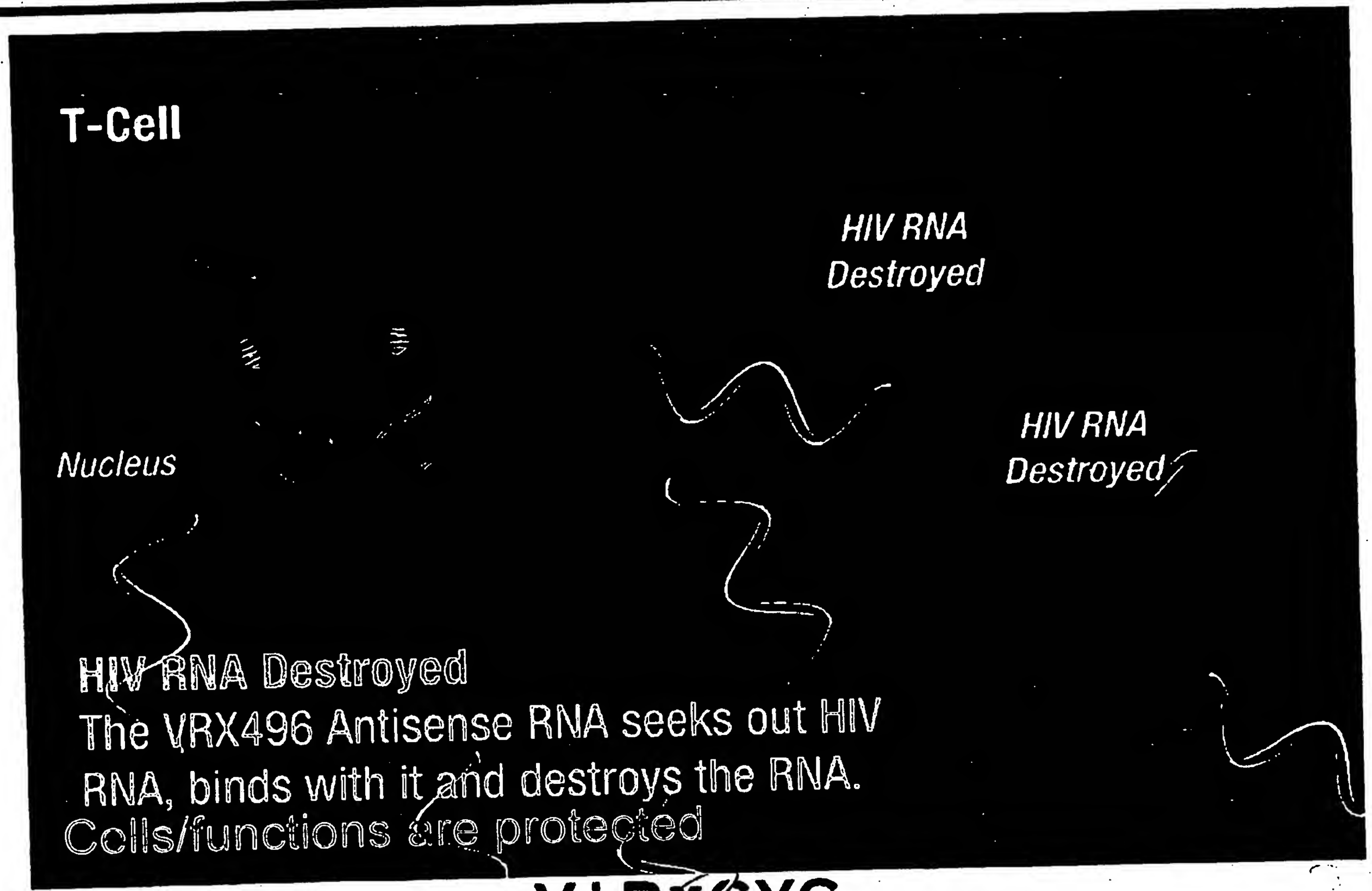
VRX496 Integrates into the Patient's T Cells



Integration
The VRX496 Antisense DNA enters the nucleus and becomes part of the cell's DNA.

VIRSYS

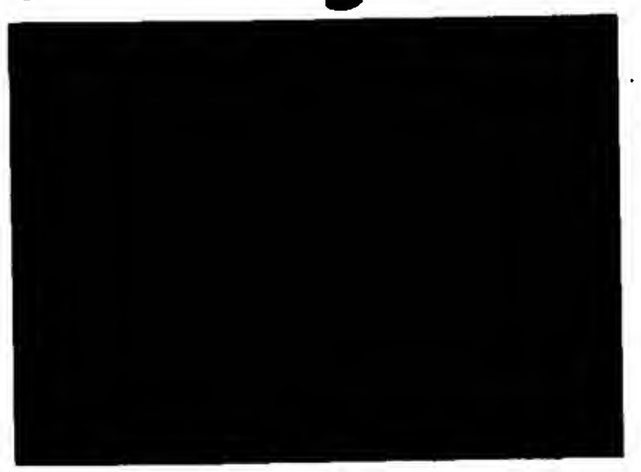
VRX496 Destroys HIV RNA Production



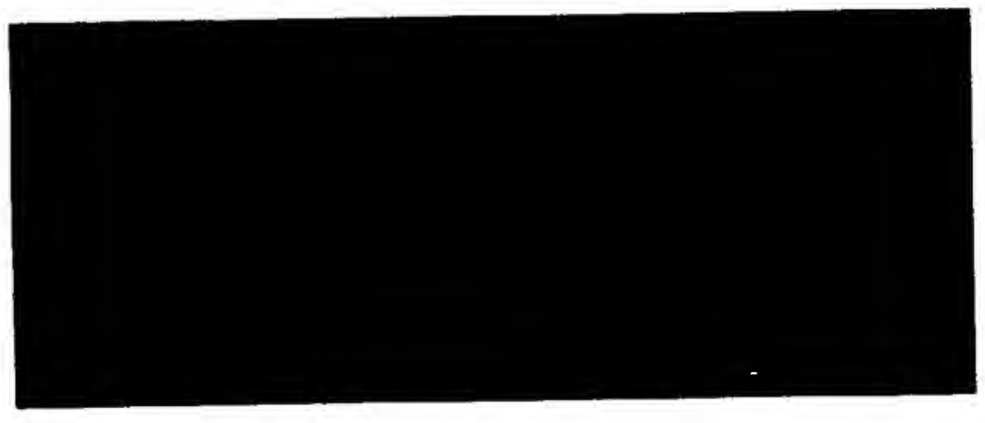
VIRxSYS

VRX496 solves the HIV resistance problem

- **Anti HIV drugs**
  - **2-8 binding sites**



- **VIRxSYS Payload**
  - **937 binding sites**



Number of Mutations Needed for Resistance	Ability to Cause Disease
Small	**High**
Large	**Low**

HIV either gets destroyed by antisense or it mutates to levels where the virus is not fit to cause disease

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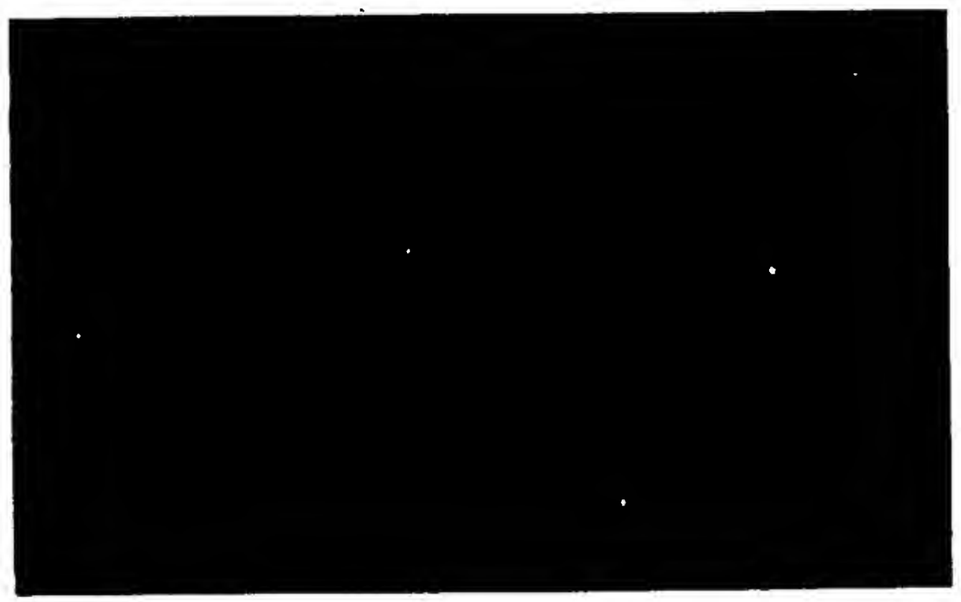
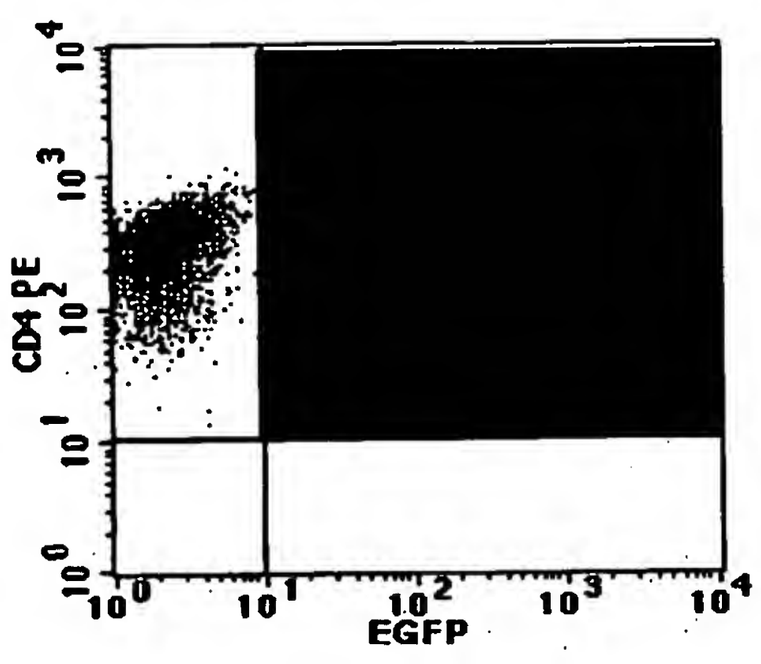
VIRxSYS

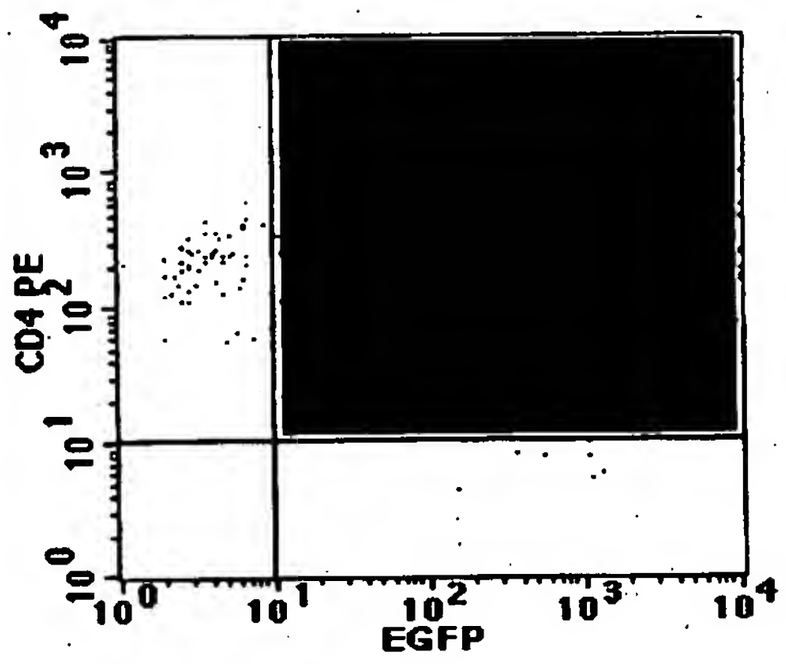
Superior Efficiency of Gene Transfer



Cluster of CD4 T cells expressing a fluorescent vector payload
(Humeau et al. Mol Ther, 2004)



0%



>99%

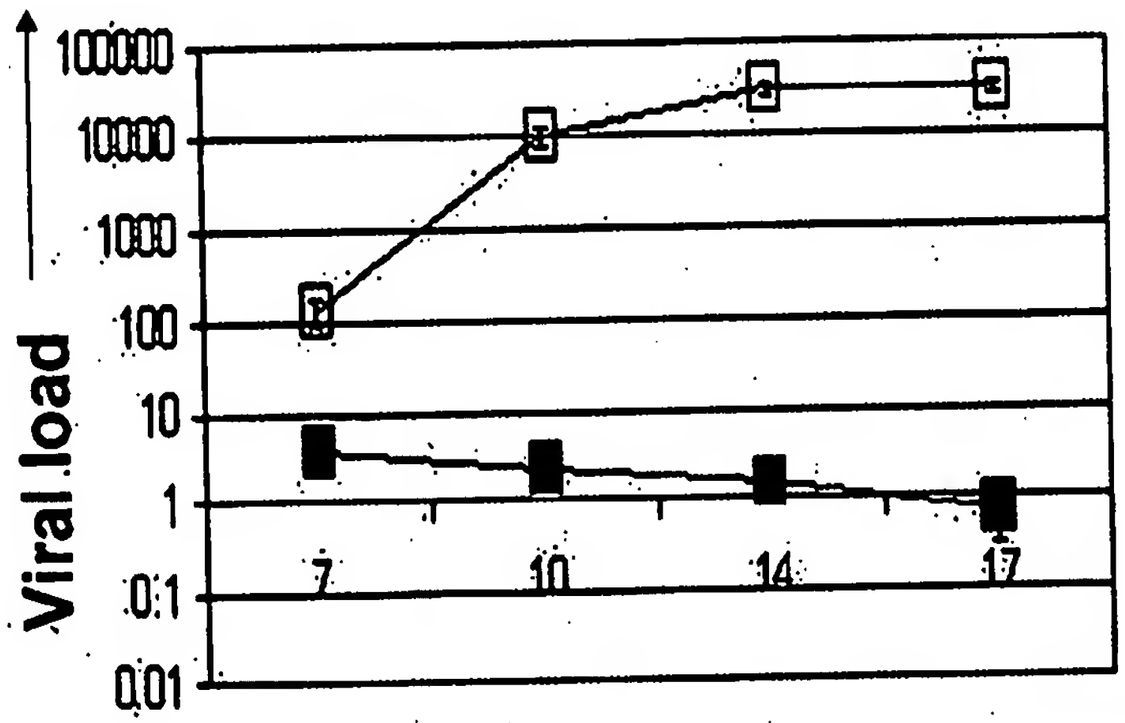
Control **+ vector- EGFP**

VIRxSYS

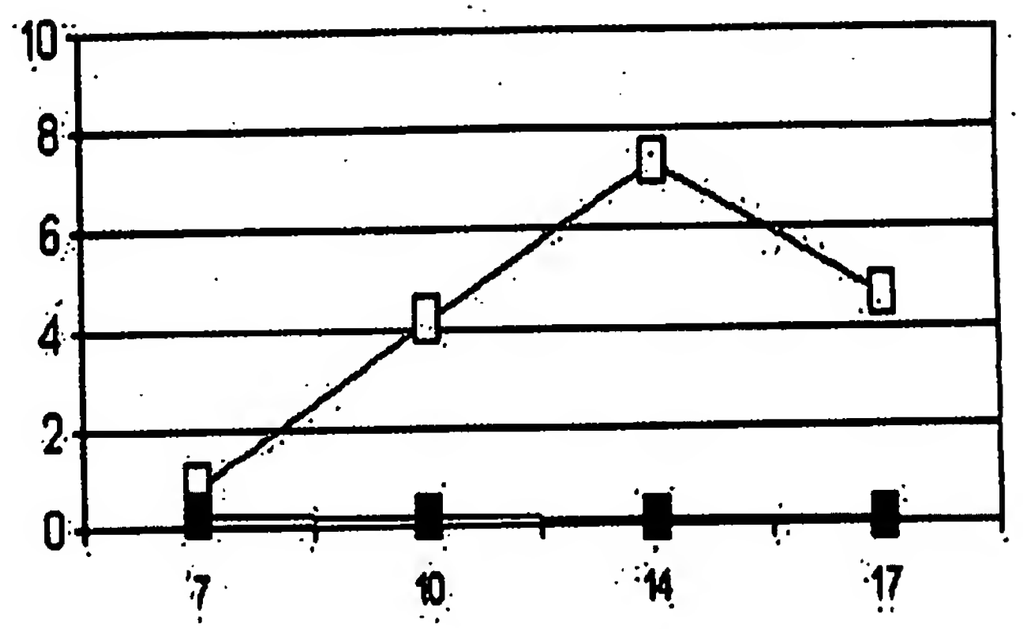
VRX 496 Suppresses the HIV Virus

J28
vl: 95,591
CD4: 288

HIV production



Percentage of infected cells

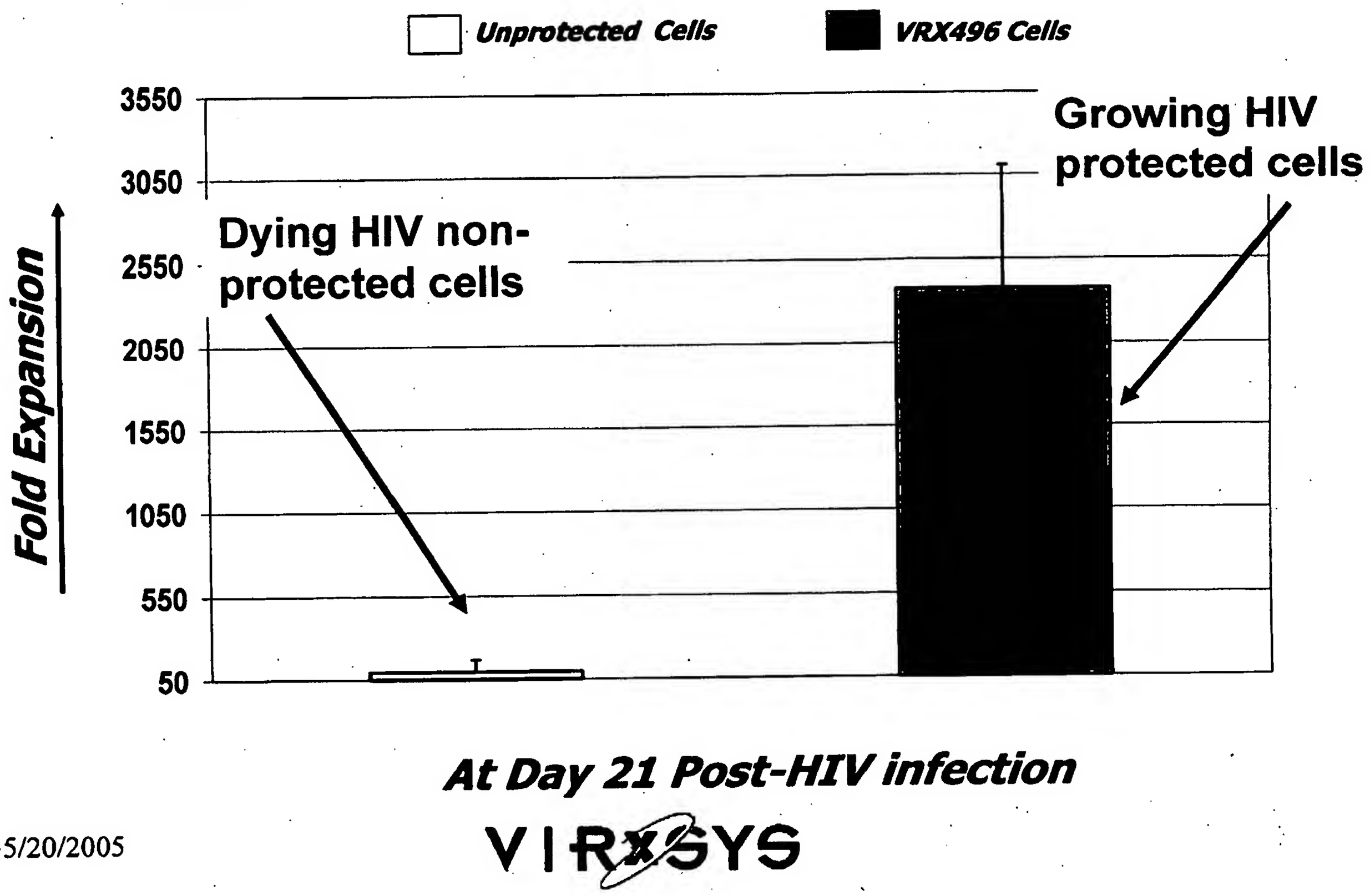


Day in Culture →

Humeau* et al, 2004 *Molecular Therapy

VIRxSYS

Survival Advantage of Vector-Modified Cells



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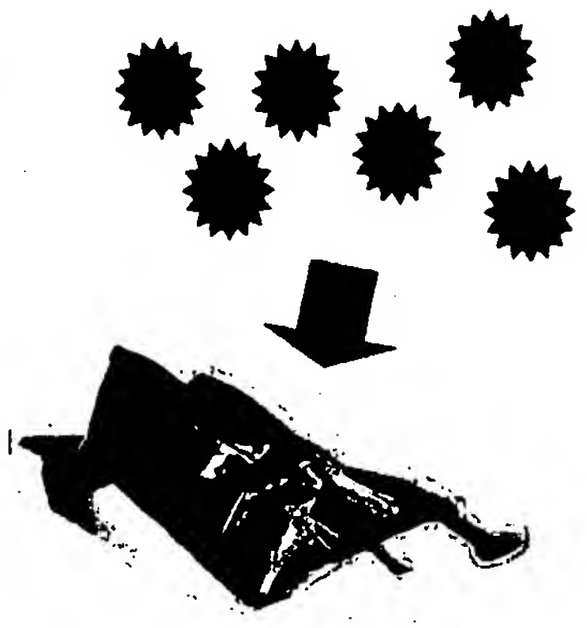
Clinical Process- autologous therapy for HIV

Patient Visit 1: Patient undergoes apheresis to isolate T cells

GOAL: T cells modified to resist HIV replication so to provide an HIV-resistant reservoir to the patient

Vector–mediated gene delivery



***Ex vivo* cell expansion**

Patient Visit 2(+): Autologous T cells modified with vector are infused to patient

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Phase I Clinical Trial to Establish Safety

UNIVERSITY OF PENNSYLVANIA
Abramson Cancer Center

INVESTIGATORS

Dr. Carl June

Dr. Rob-Roy MacGregor

Enrollment

- 5 patient trial

- Serial enrollment for safety

Patient Criteria

- No good treatment options left

- Failed 2 regimens of HAART drug therapy

- Viral loads > 5000

- CD4 T cell counts 150 - 500

VIRXSYS

Study Subject Characteristics

Table 1. Baseline characteristics of HIV subjects

Characteristics	#1 RB	#2 JFJ	#3 RAG	#4 AJ	#5 JF
Age	41	44	40	27	45
Gender	M	M	M	M	M
Ethnic Group	Caucasian	Caucasian	African American	African American	Caucasian
Mean viral load	188,500	54,100	46,150	54,213	19,972
Mean CD4 counts	228	316	241	308	220
HIV Infection (Yrs)	15	15	15	10	9
Discontinued Therapy	6 NRTI +2 NNRTI +5 PI	5 NRTI +4 PI	6 NRTI +1 PI	4 NRTI +2 NNRTI +1 PI	4 NRTI +1 NNRTI +1 PI
Current Therapy	2 NRTI +2PI	3 NRTI +1 NNRTI +1 PI	None	2 NRTI +1 NRTI	2 NRTI +1 PI

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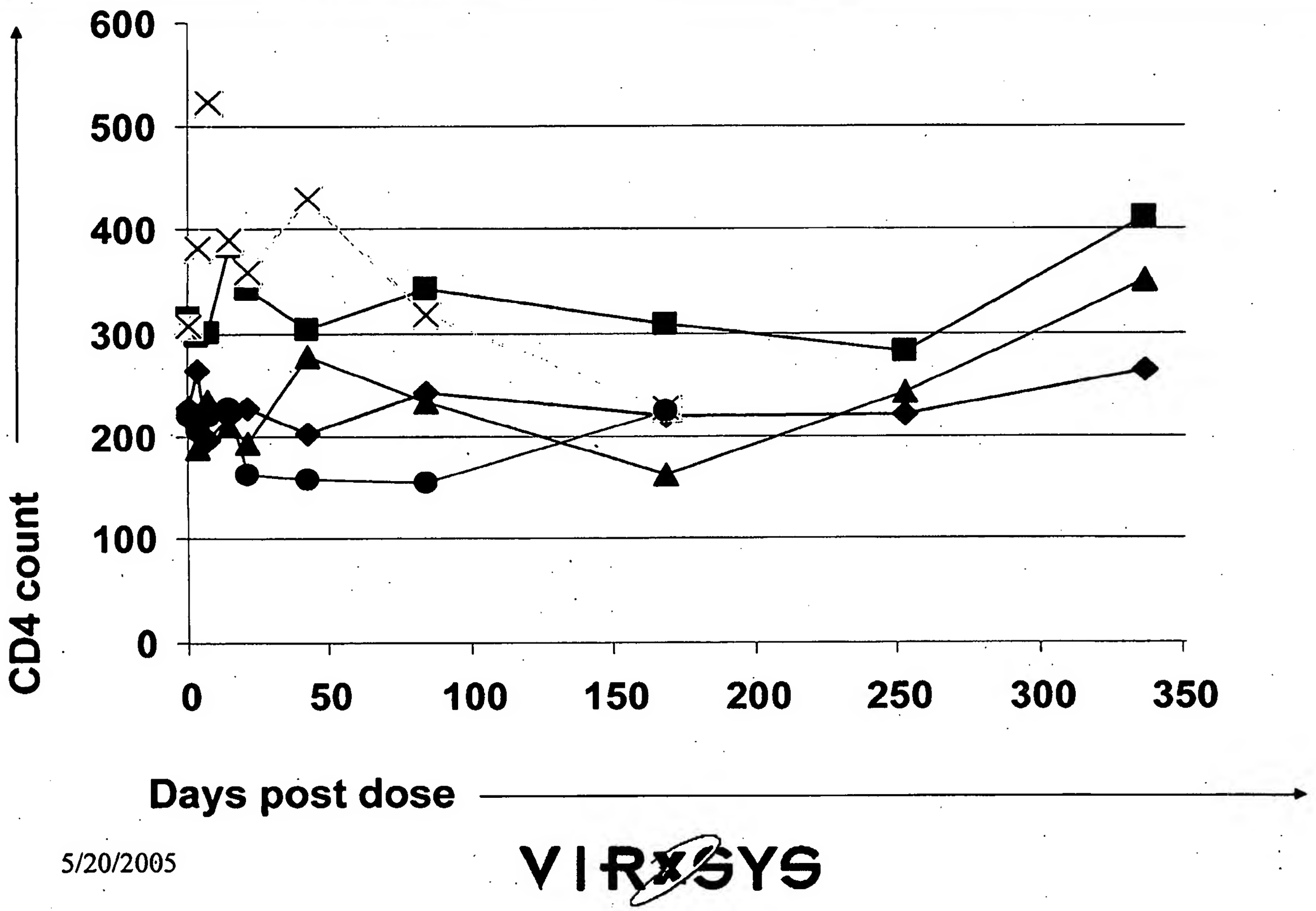
CD4 Counts



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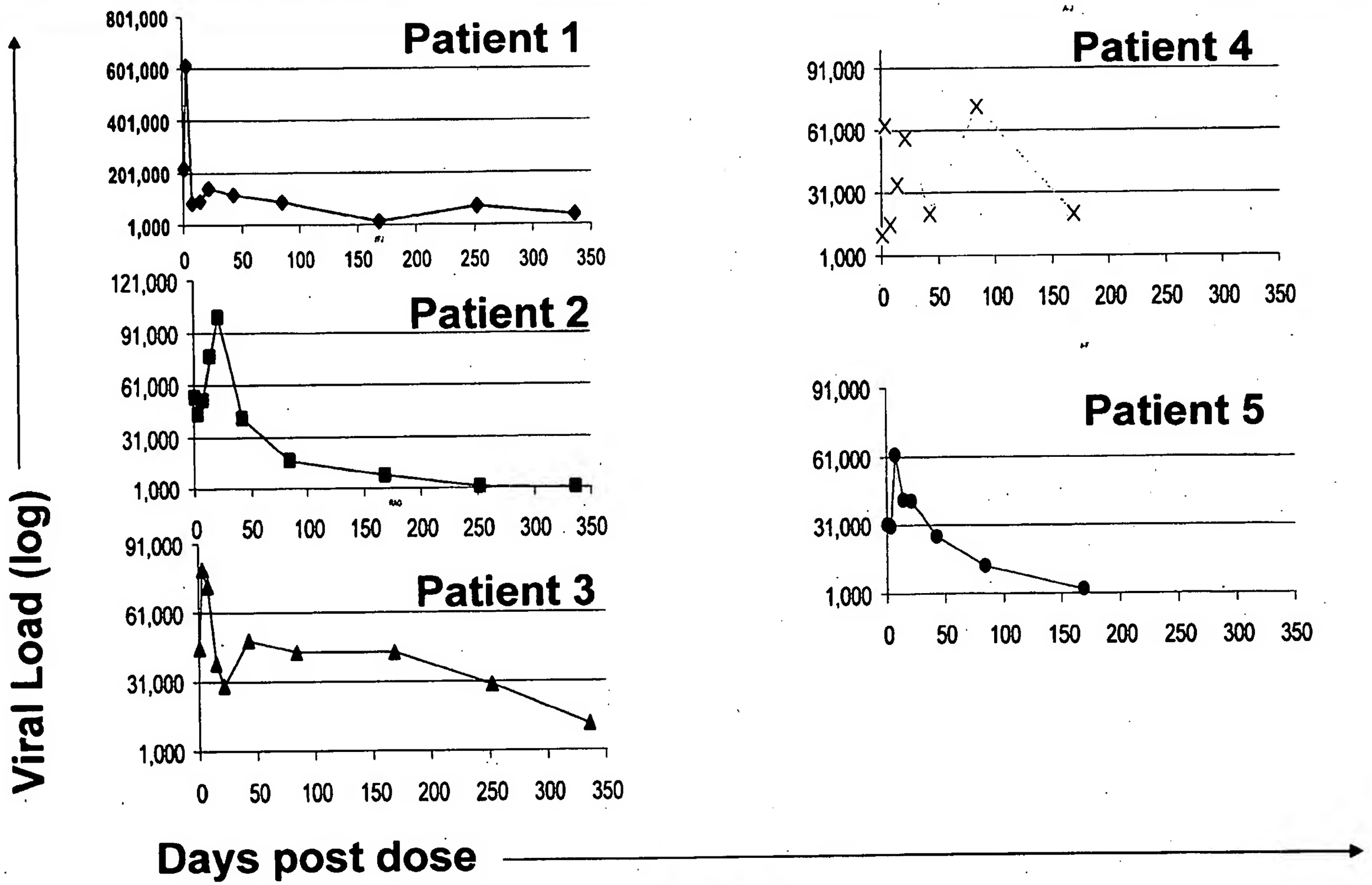
Clinically Meaningful Viral Load - Detail



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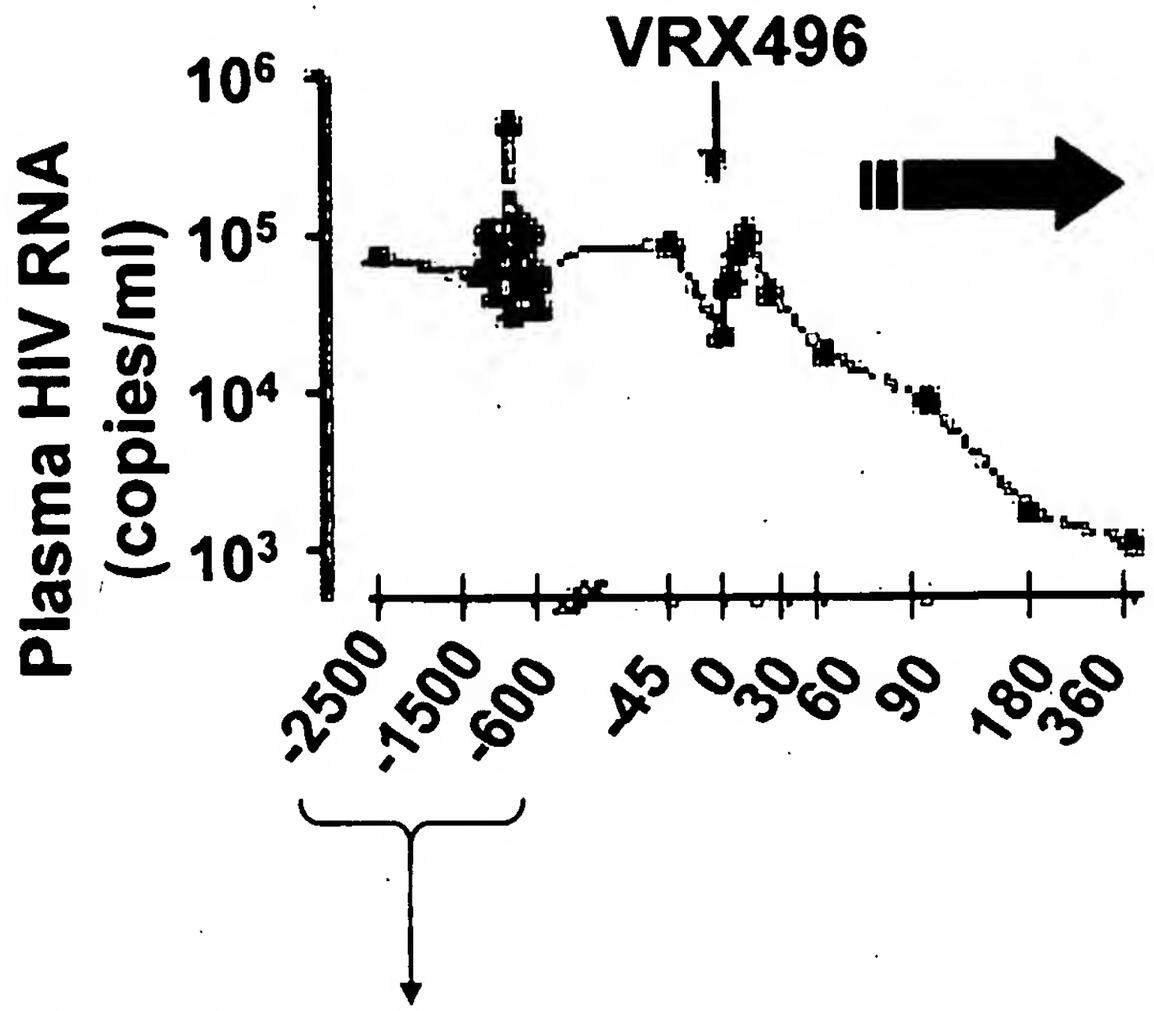
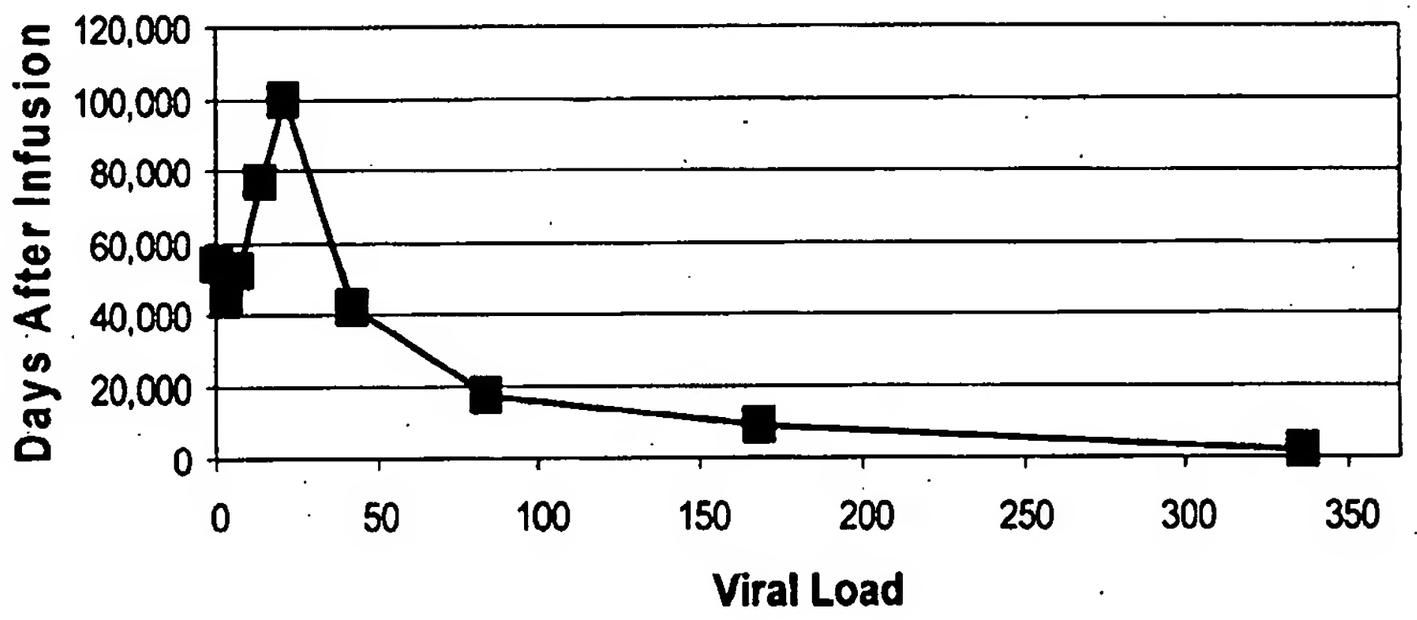
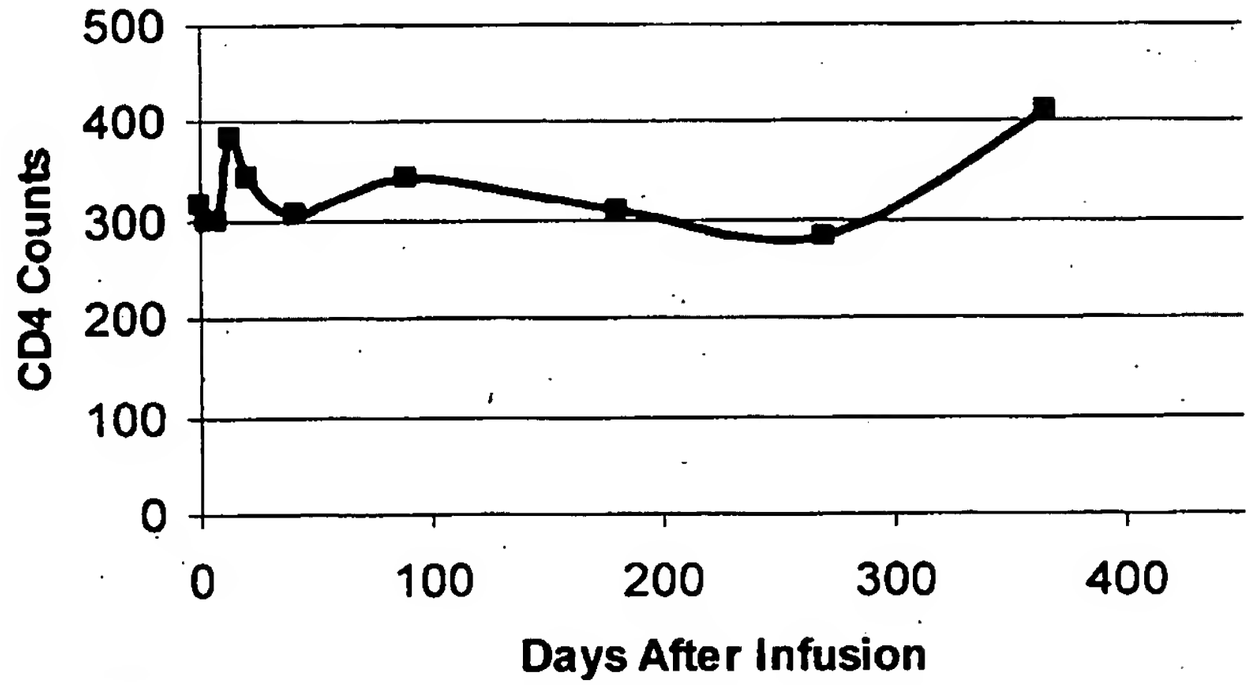
The "Philadelphia" Patient-Detail



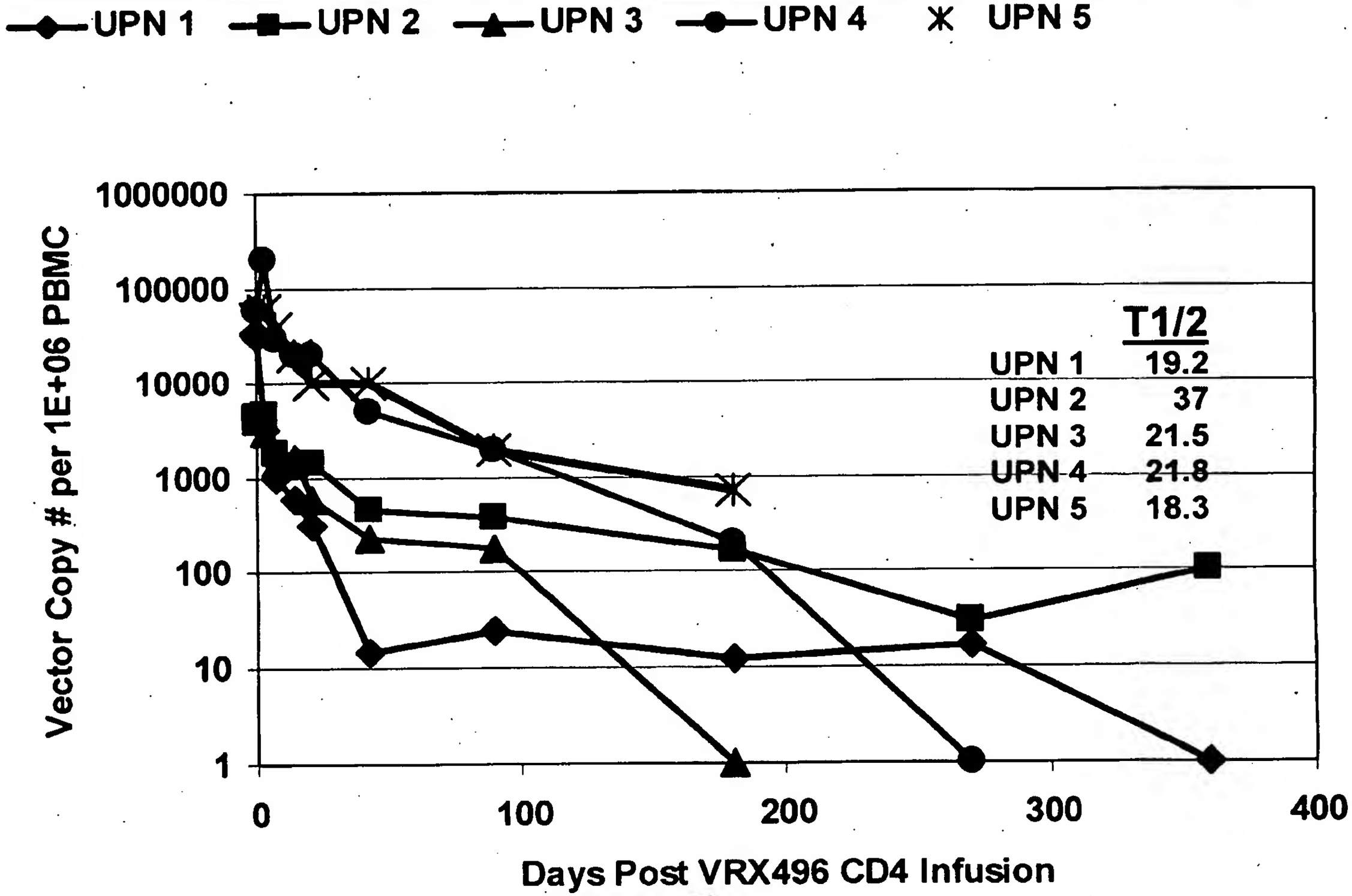
Past 7 years of viral load
Drug failure





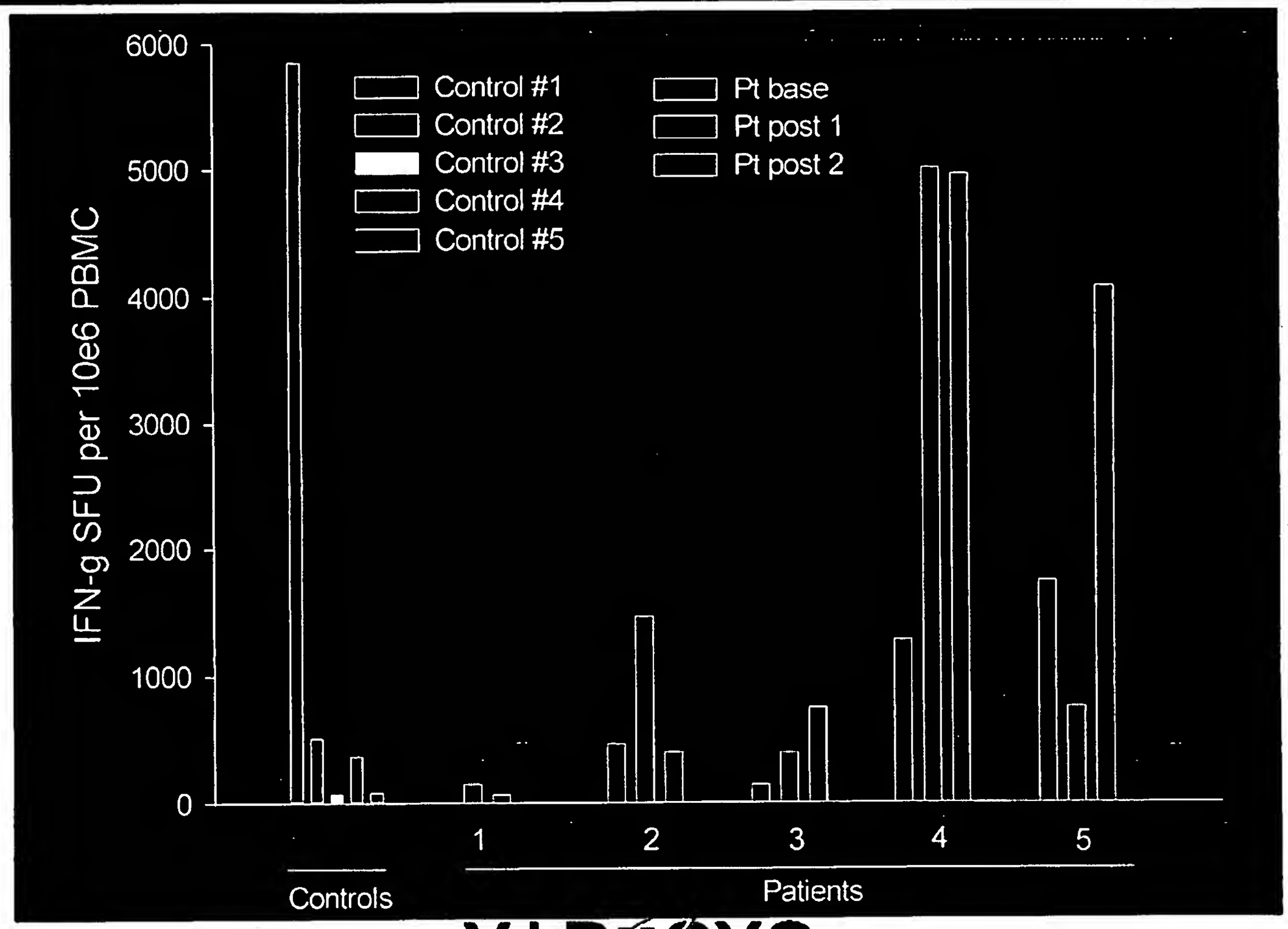
VIRxSYS

Sustained Engraftment and Persistence of VRX496 Modified CD4 T Cells



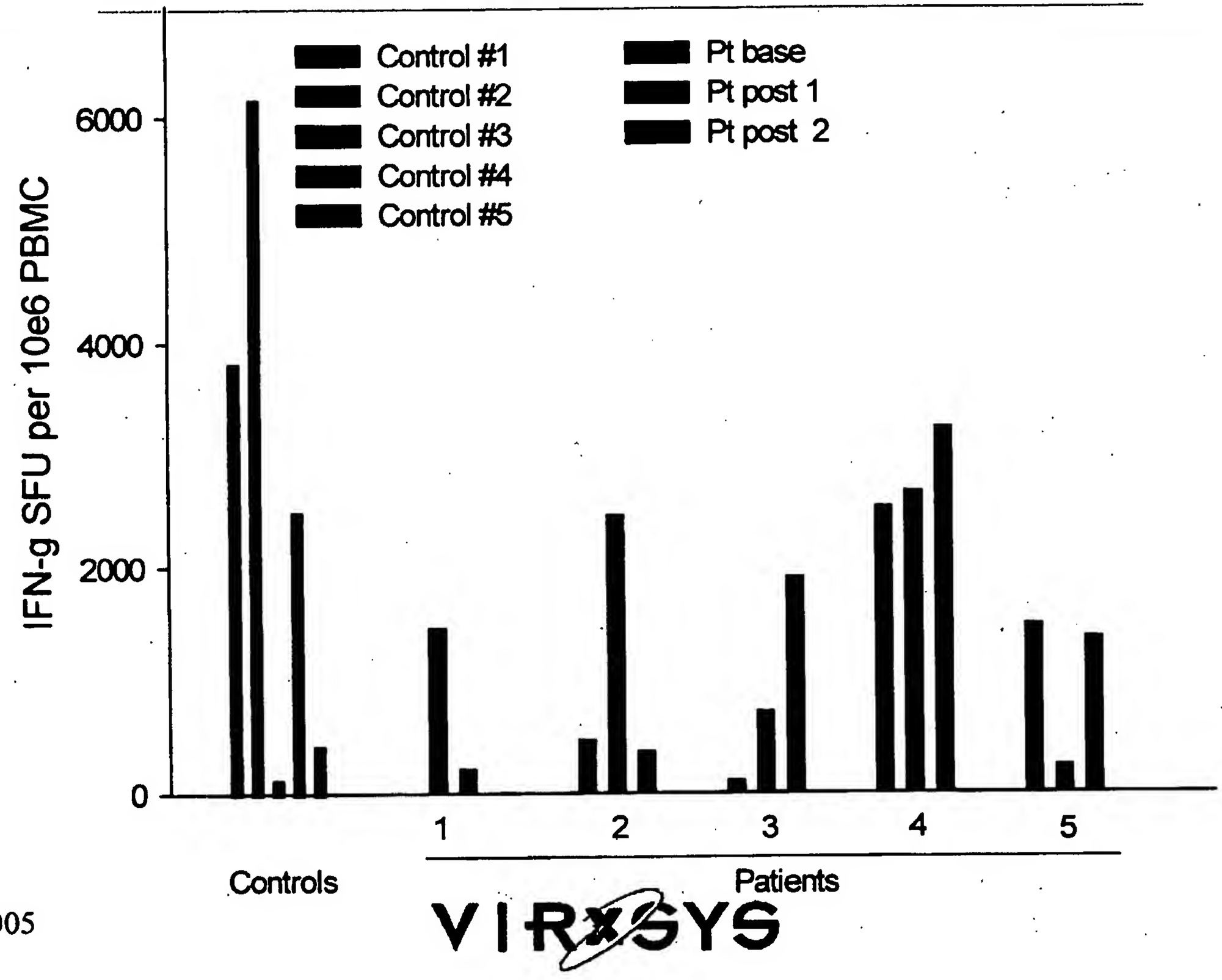
VIRxSYS

Immune Function Analysis: IFN-γ
ELISPOT -- Env



VIRxSYS

Immune Function Analysis: IFN-γ
ELISPOT -- gag



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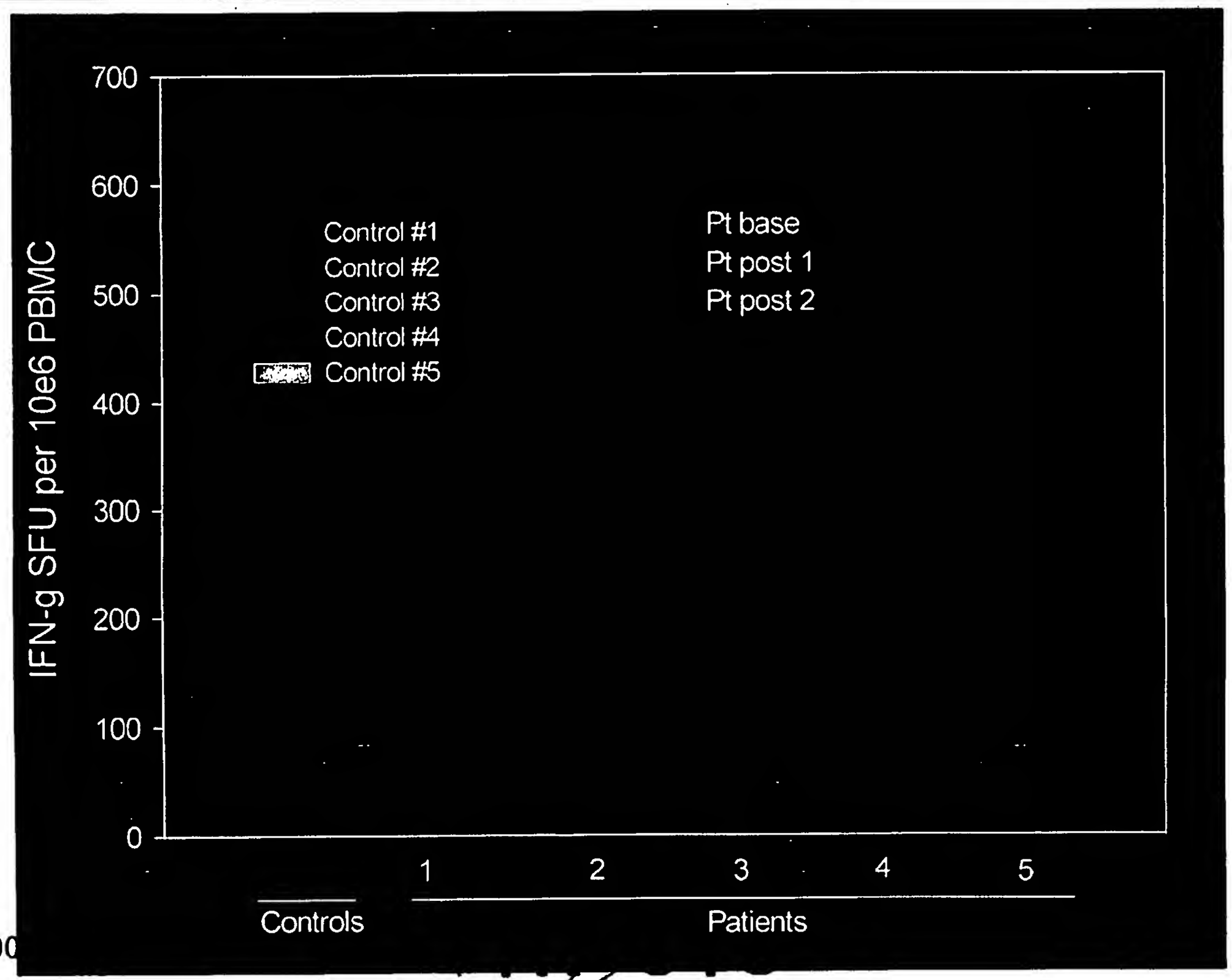
VIRxSYS

Immune Function Analysis: IFN-γ ELISPOT Diphtheria

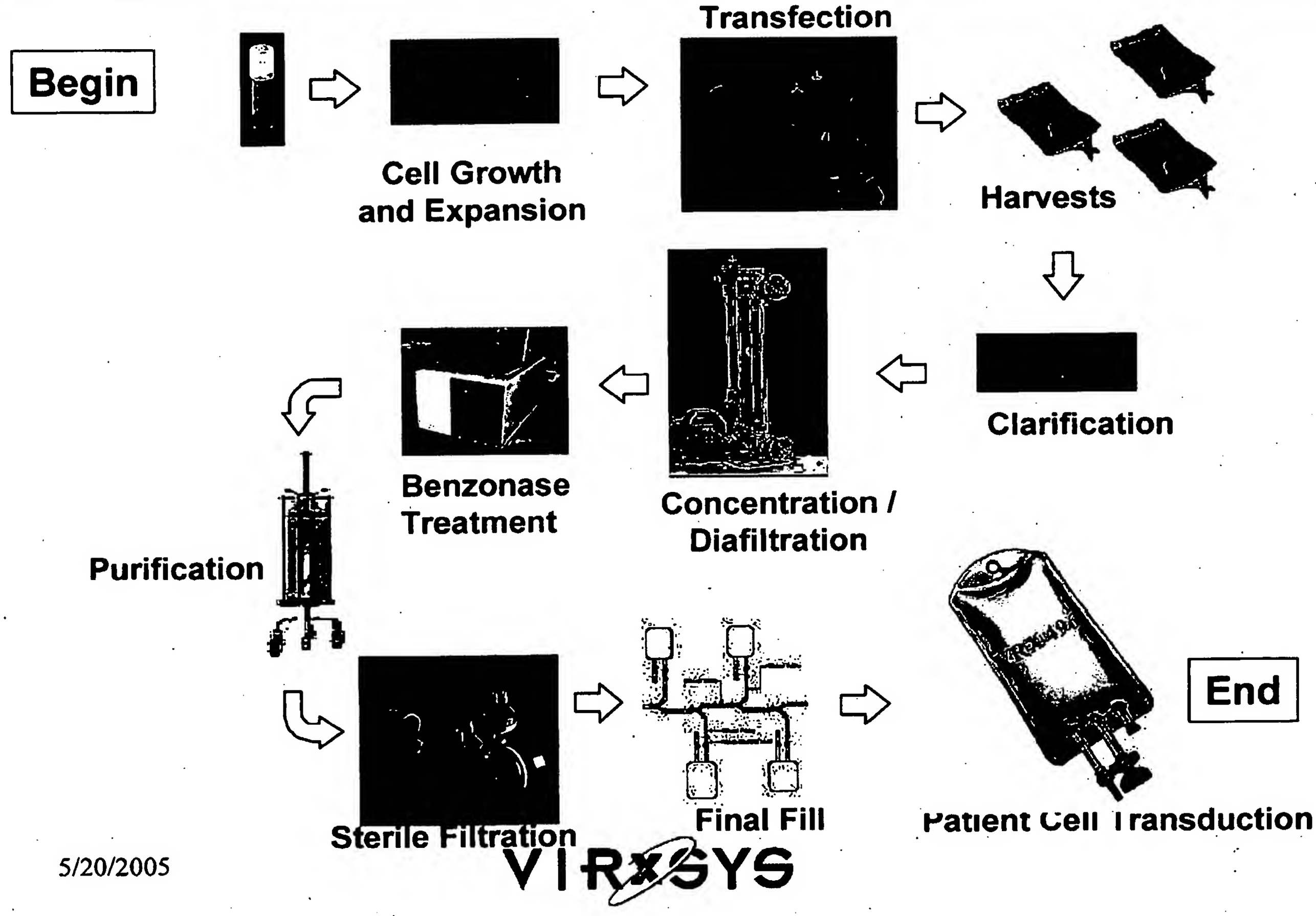


What's next?

- **Phase II Efficacy Clinical Trial**
  - Dose tolerability and dose finding trial
  - 4 and 8 dose cohort of 5-10 x 10^{10} cells
  - Multi-center trial

- **Drug Holiday and VIRxSYS Therapy**
  - Multidosing (6 doses)
  - University of Pennsylvania, Dr. Carl June
  - Investigating an extension of drug holiday by VRX496 modified cell transfer

Current Production Process

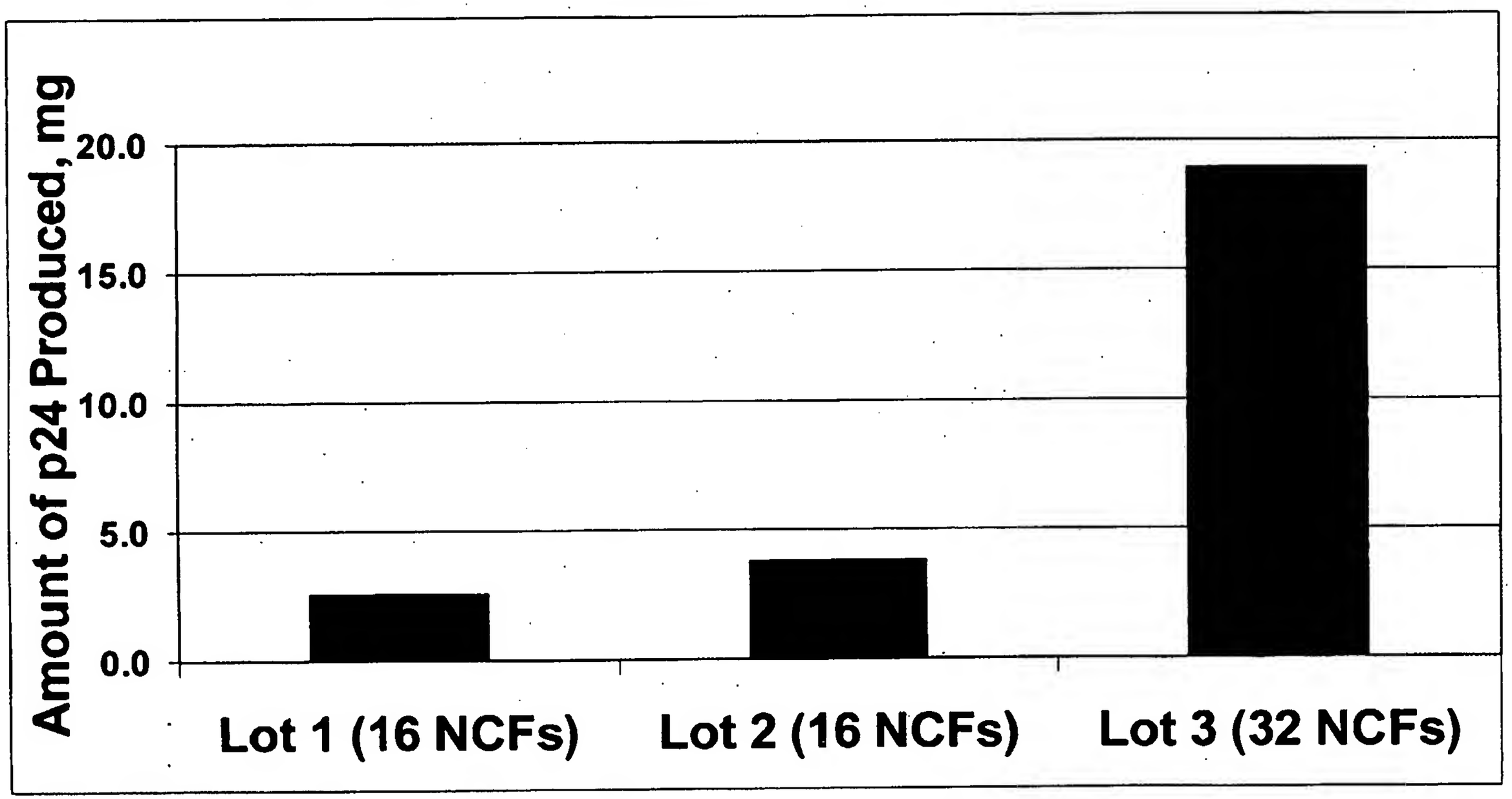


VIRxSYS

Adjustments for Increased Vector Production and Yield

Media	with10% FBS	with 5% FBS
Transfection	individual cell factory per bag	8 cell factories per bag
Nunc Cell Factories	16	32
Harvest Collections	2	3
Harvest Volume	~35 L	~ 105L
Collections Mode	Individual NCF	8 NCF per bag
Clarification Filters	2 ft^2	5 ft^2
Concentration	~40 fold	~ 100 fold
Chromatography	4 separate 1L columns	2 serially connected 5L columns
Final volume	~1.2 L	~ 2.5 L

More than 2-Fold Increase in Overall Yield by Doubling Cell Factories for Production



VIRxSYS

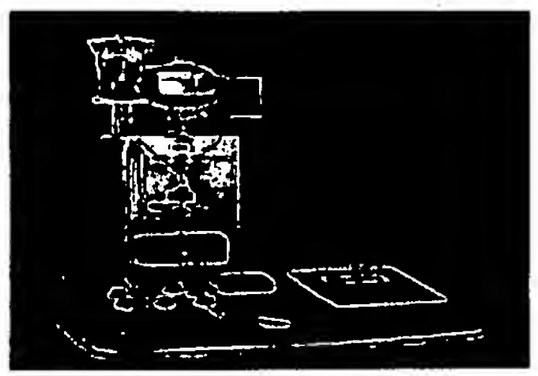
Autologous T Cell Manufacturing Process

FROZEN APHERESIS

CYTOMATE / BAXTER

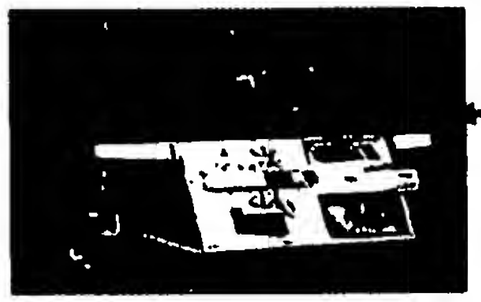
Wash

MACS CD8 INCUBATION

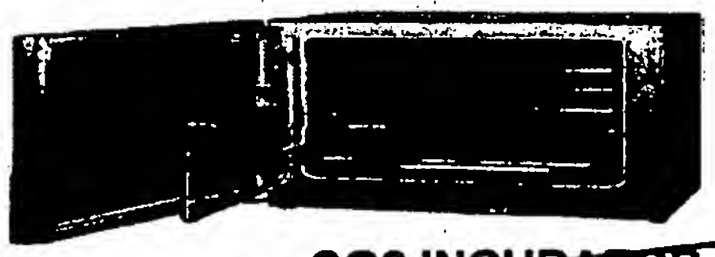


CLINIMACS / MILTENYI

CD4 Enrichment

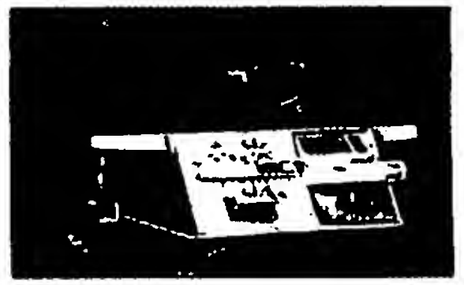


Buffer Exchange

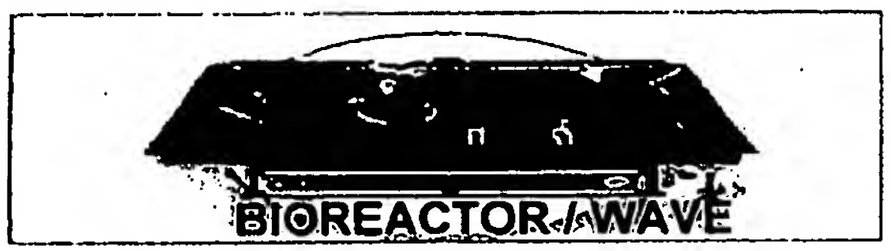


CO2 INCUBATOR

Transduction: 3 Days



Vector Wash and Medium Exchange



Expansion 7 to 10days

Medium Exchange/Wash,
Beads removal,
Formulation
Cryo-preservation

LIQUID NITROGEN STORAGE
TANK / MVE

5/20/2005

RxSYS

S27
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Summary of Major Manufacturing Changes Made Between Phase I and Phase II

Manufacturing Change	Description
Change in Facility	Change to VIRxSYS Cell Processing Facility
Initial Wash of Apheresed Cell Product to Remove Plasma	From ficoll wash to CytoMate (Miltinyi) wash
CD4 Purification Process	Changed from CD8 depletion to CD4 selection
Cell Washing throughout Process	Changed from washes using Cobe (Baxter) to washes using Cytomate
Cell Expansion	Using WAVE bioreactor in contrast to expanding cells in plastic bags
Transduction	Using 2-fold less vector

VIRxSYS

CD4+ T Cell Purity: Phase 1 Cell Product Versus Phase 2 Development Lots

UPenn Phase 1 Cell Product	VIRxSYS Phase 2 Development Lots
CD4+ Cells: 56%, Abs. 3.06 x 10^8 (52 % recovery)	CD4+ purity: 97.62%, Abs. 3.419 x 10^9 (47.7% recovery)
CD4+ Cells: 52.2%, Abs. 4.38 x 10^8 (48% recovery)	CD4+ purity: 97.4%, Abs. 1.48 x 10^9 (40.5% recovery)
CD4+ Cells: 23%, Abs. 1.67 x 10^8 (26% recovery)	CD4+ purity: 91.77%, Abs. 2.05 x 10^9 (48.8% recovery)
CD4+ Cells: 33.4%, Abs. 2.93 x 10^8 (30% recovery)	
CD4+ Cells: 19.5 %, Abs. 1.77 x 10^8 (23% recovery)	
Average CD4+purity: 36.82% (range 19.5 to 56%)	**Average CD4+ purity: 95.60% (range 91.77 to 97.62%)**

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VIRxSYS

Transduction Efficiency : Phase 1 Patient's Cells Versus Phase 2 Process Development Lots

Phase I Cell Product		Phase II Development Lots	
Subject Study ID	**Vector copy number per cell**	**Process Run #**	**Vector copy number per cell**
001-022 J-K	1.20	1	2.80
001-017 A-J	4.10	2	1.19
001-010 RAG	0.98	3	1.48
001-001 JFJ	1.80		
001-002 R-B	2.3		
Average	**2.08**		**1.82**

VIRxSYS

Expansion Efficiency: Phase 1 Patient's Cells Versus Phase 2 Process Development Lots

Phase I Cell Product		Phase II Development Lots	
Subject Study ID	**Total Cells / Fold Expansion**	**Process Run #**	**Total Cells / Fold Expansion**
001-022 J-K	$15.8x10^9$ / 65	1	$52.3x10^9$ / 28.6
001-017 A-J	$20.6x10^9$ / 40	2	$104x10^9$ / 58.8
001-010 RAG	$6.8x10^9$ / 25	3	$96.6x10^9$ / 63
001-001 JFJ	$11.5x10^9$ / 32	4	$87.5x10^9$ / 63.2
001-002 R-B	$15.2x10^9$ / 66		
Average	**$14x10^9$ / 45.6**		**$85.1x10^9$ / 53.4**

VIRxSYS